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PROCEEDINGS OF

THE ROYAL SOCIETY.

SECTION B —BIOLOGICAL SCIENCES

Experimental Researches on Vegetable Assimilation and Respiration XIX —*The Effect of Variations of Carbon Dioxide Supply upon the Rate of Assimilation of Submerged Water Plants*

By W O JAMES Ph D St John's College Cambridge

(Communicated by Dr F F Blackman, F R S —Received January 30, 1928)

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INTRODUCTION AND METHOD

Observations made hitherto by different workers on the relation between concentration of carbon dioxide and the rate of its assimilation by plants are not entirely harmonious, and it seems possible that much of the apparent disagreement may be due to the differences in the experimental conditions which have been used. In recent work on aquatic plants, bicarbonate solutions have been freely utilised as method of supplying carbon dioxide, instead of the carbon dioxide solutions of earlier workers. For these methods to be directly comparable with one another it must be assumed that only carbon dioxide present in the free form is assimilable by the plant, and that HCO_3 ions and undissociated salts ($NaHCO_3$, etc.) are not available. This assumption has been supported by Nathansohn, 1907 (13), Benecke, 1921 (3), and Wilmott, 1921 (23), but opposed by Angelstein, 1911 (1), Osterhout and Haas, 1918 (14), and Ruttner, 1921 (16).

The present paper records an attempt to reinvestigate this problem by means of a method which allows direct comparisons to be made between these two types of solution. Each solution has been investigated over a range of concentrations, and using varying intensities of light. Attention has also been given to the rate of flow of the solutions over the assimilating plant. In this way a collection of assimilation values under precisely defined conditions has been built up, which helps a closer understanding of the interaction of factors in this process, and enables us to get a clearer picture of the essential differences between carbon dioxide and bicarbonate solutions as media for assimilation.

Widely contrasted methods used in the past are those of Blackman and Smith (6) and Harder (10). The former used solutions of carbon dioxide flowing continuously over the plant from a reservoir, while the latter used small volumes of bicarbonate solutions which remained at rest. The relations of assimilation to varying concentrations of the solutions recorded by these two methods appeared to have little in common. The method of Harder, 1921 (10), is simple and easily copied, and some preliminary experiments done at the outset of this investigation showed that his results were readily repeatable. This being established, it was proposed to devise an apparatus that would permit closer investigation of the Harder type of curves.

Several systems were considered and experimented with in a preliminary manner, but the only principle which offered any considerable prospect of success was that used by Blackman and Smith in 1911 (6), which allowed a continuous current of solution to be passed over the plant material. While the maintenance of approximately constant gas concentrations was secured by their method, the modifications required for this special research, in which oxygen production and not carbon dioxide consumption was to be measured, developed virtually a new apparatus and practice. Some description is, therefore, necessary.

As finally constituted the apparatus consisted of three principal parts: (*a*) a plant chamber, (*b*) a reservoir supplying the inflowing solution, and (*c*) a receiver which collected the outflow for analysis.

(*a*) *The Plant Chamber.*

In the initial experiments the original chamber described and used by Blackman and Smith was utilised. This, however, was found to be unsatisfactory for the present purpose, as the wax with which it had to be lined absorbed a measurable quantity of oxygen during the experiment. A chamber, entirely of glass, was therefore constructed, and contact of the solution with rubber was reduced to a minimum throughout the apparatus.

The new chamber was designed as follows: A flat-sided glass flask, about 14 × 6 × 2 cms. was fitted with an inlet tube of medium bore, which reached to the far end. The tip of the tube was sealed off, and four small lateral holes of equal size were pierced near the tip. At the stopper end the tube was sealed into a short tube of wider bore, which had an outlet on its upper side. The wide tube fitted into the neck of the chamber by a rubber stopper, the narrow surface of which, in contact with the solution, was coated with a thin film of high-melting-point paraffin wax. The figure (fig. 1, p. 5, G) shows how the inlet and outlet tubes were arranged for convenient connection with the other parts of the apparatus.

By tests with a slow flow of tinted water into the colourless water of the chamber, it could be demonstrated that the incoming solution displaces the latter in a smooth, steady flow from base to neck of the chamber. Constant conditions of temperature were obtained by immersing the chamber in a thermostat with a toluol regulator, which normally maintained the temperature constant within 0·1° C. Light was supplied by incandescent electric lamps, one of 1500 c.p. and another of 300 c.p. The intensity was regulated by sliding them up or down a tall stand, and was measured by means of a Moll thermopile, enclosed in a glass jar and immersed beside the plant chamber. Between the lamp and the thermostat tank a glass-bottomed dish was supported, and a current of cold water passed through it to cut off the heat given out by the lamp. The level of the water in the bath was kept constant by an automatic siphon, so that its absorption of radiation should not fluctuate.

(b) *The Reservoir of Inflowing Solution.*

It was a great gain to have duplicate reservoirs of solution, arranged so that either could be drawn upon by simply turning a tap (fig. 1, e). Each reservoir consisted of an inverted bell jar (fig. 1, B and C), fitted below with a rubber cork pierced for three tubes, one connected through the three-way tap (e) with the other parts of the apparatus, one for a supply tube (fig. 1, a) for filling the reservoirs, and the third for drawing off samples for analysis (fig. 1, b and c). The upper end of the bell-jar had a ground edge and was closed by a glass plate and a vaseline joint. The plate had a cork, fitted with inlet and outlet tubes through which a current of gas from a pressure cylinder could be kept flowing, when desired. When carbon dioxide-free water was put into the reservoir, a soda-lime tube was attached, so that air, freed from the gas, could be sucked in by the fall of the liquid level as the solution was used up. In most cases it was not found practicable to ensure a strict equilibrium between the dissolved gases in the experimental solutions and the atmosphere above them in the reservoirs. In some cases this was due to the smallness of the amount of dissolved carbon dioxide used. When the water was actually carbon dioxide free, or in equilibrium with the atmospheric concentration, no difficulty arose on this account, but even then the oxygen concentration, which could not be used at "air equilibrium," introduced a further complication.* To reduce loss or gain of these significant gases by diffusion across the interphase surface, a paraffin-wax float was made to fit inside each reservoir. These floats rested on the solution and rose and fell with its surface, reducing the contact of air and liquid to a small ring round their edges, as the reservoirs had been selected for their true cylindrical form

Trial experiments with the final form of the apparatus showed that adequate protection had been attained against extraneous diffusion changes. Direct titration of the oxygen content of solutions standing in the reservoirs showed no drift with time, and prolonged series of constant assimilation results indicated that the carbon dioxide content also kept constant.

After leaving the reservoir on its way to the chamber the solution was passed through a U-tube (fig. 1, E), designed to maintain a constant rate of flow. The farther arm of this was wide, and opened at the top into a small inverted cup with a narrow opening above and an overflow tube passing through the rubber cork forming its bottom. About 6 inches below the open end of the U-tube a narrow side tube was fitted, which led to the plant chamber through a length of capillary (fig. 1, F). The three-way tap controlling the liquid supply was turned on just sufficiently to cause the solution to overflow slowly at the open end of the U-tube and so pass away through the waste tube to a flask. This arrangement ensured a constant head of water, independent of the falling level in the reservoir, and only needed occasional adjustment of tap e for economical working.

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* Nitrogen equilibria were not considered important, except that experience showed that if the nitrogen content of the water were allowed to get too high, free bubbles were formed in the apparatus, which would carry off some oxygen and carbon dioxide with them.

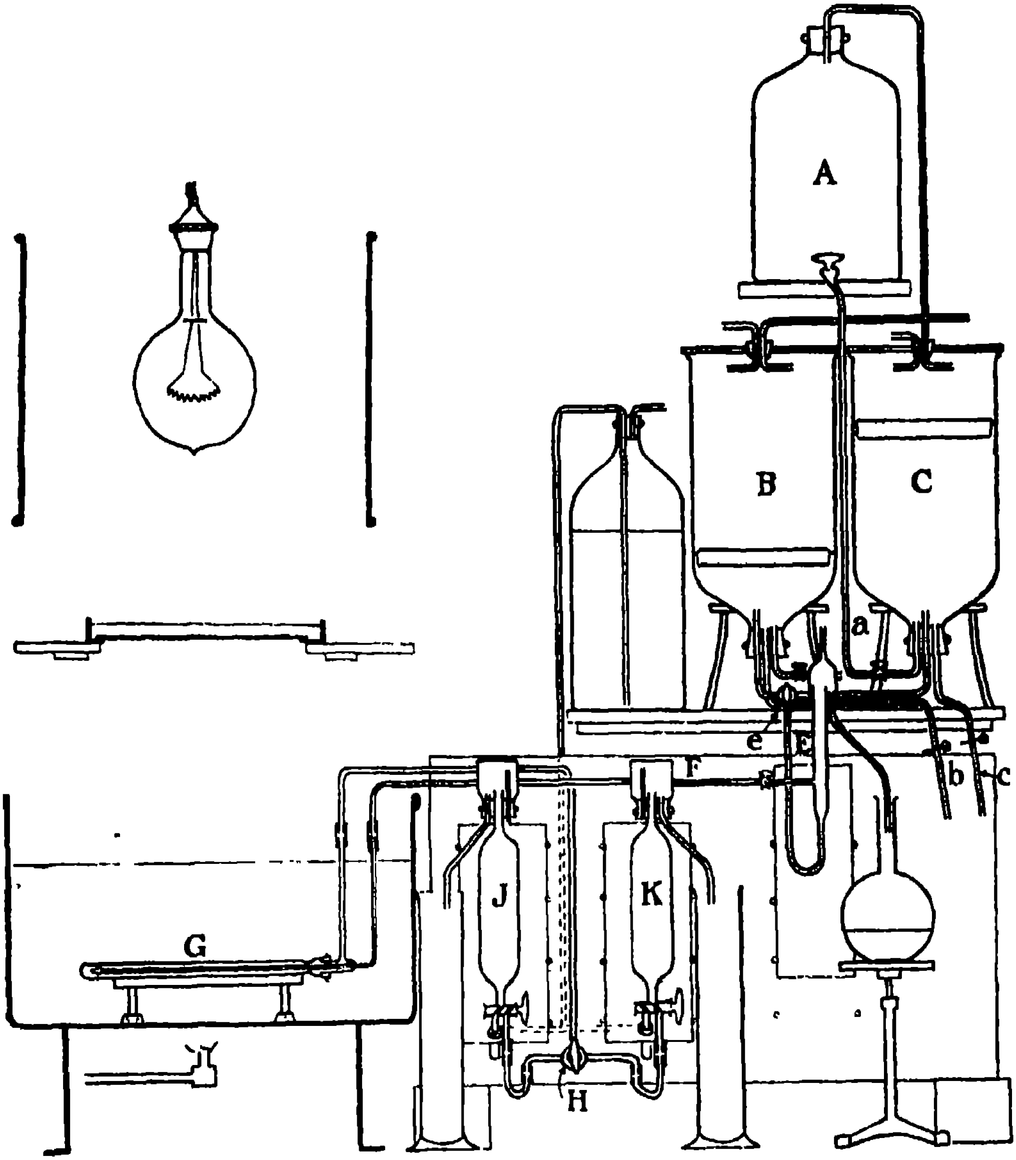

Fig. 1.—The Assimilation Apparatus (for lettering see text).

(c) *The Receiver of the Outflowing Solution.*

The solution supplied by the reservoirs was collected, after passing through the plant chamber, in a pair of pipettes, each of 200 c.c. capacity. The arrangement was similar, with only minor modifications, to that used by Blackman and Smith. The pipettes were individually mounted on vertical slides and connected to the three-way tap (fig. 1, H), leading from the plant chamber, by glass tubes with rubber connections allowing a small degree of mobility. The overflow arrangements at their upper ends were covered in (fig. 1, J and K) to minimise the chances of the entry of dust and bacteria.

The Solutions.

Solutions of varying strength of carbon dioxide and sodium bicarbonate were employed. They were made up in distilled water which was freed from atmospheric gases by boiling in 2-litre flasks. After boiling it was allowed to cool in the flasks with soda-lime tubes inserted in their stoppers. Besides freeing the water from carbon dioxide it was also of fundamental importance to keep down the oxygen content, so that, even with fairly high production of oxygen in the assimilation, no free bubbles of gas were formed in the chamber, which would entirely invalidate the experiment. Also variable oxygen-content of the inflowing solution would be a disturbing factor on account of its effect on the respiration rate. To control the gas contents of the prepared water it was stood over night in a large bottle maintained at 25° C. with a current of carbon dioxide-free air bubbling through it.

To prepare the carbon dioxide solutions, about half a litre of distilled water was thoroughly mixed on a mechanical shaker with well-washed carbon dioxide from a Kipp. This concentrated solution was titrated by the baryta method and a suitable amount diluted to the concentration required with the water prepared as described. The solutions were made up in 2-, 3-, or 4-litre lots, usually about 10 to 30 c.c. per litre of the strong solution being required. Sodium bicarbonate solutions were prepared in similar quantities, simply by dissolving the amount of salt required according to the calculations in the following paragraph, in the specially prepared water. The measuring flask or bottle was then connected with one of the reservoirs of the apparatus (fig. 1, A) and the solution allowed to run into it.

The Concentration of Free Carbon Dioxide in Solutions of Sodium Bicarbonate.

Sodium bicarbonate, in solution, gives rise to the following equilibria :—

$$NaHCO_3 \rightleftharpoons Na + HCO_3 \quad (1)$$

$$HOH \rightleftharpoons H + OH \quad (2)$$

$$HCO_3 + H \rightleftharpoons H_2CO_3 \quad (3)$$

$$HCO_3 \rightleftharpoons H + CO_3 \quad (4)$$

$$Na + OH \rightleftharpoons NaOH \quad (5)$$

The quantity of HCO_3 ion involved in the fourth equation is so small as to be negligible from the present point of view, and since it is not possible to distinguish between carbon dioxide as carbonic acid and as gas in solution, the quantity of " free carbon dioxide " is usually regarded as the amount corresponding to the right-hand side of equation (3). It is related to the original concentration of sodium bicarbonate as follows :—

If k_1 is written for the " first dissociation constant " of carbonic acid, the square brackets indicating concentration,

$$\frac{[H]\,[HCO_3]}{[H_2CO_3]} = k_1, \quad (6)$$

and if the degree of dissociation of sodium bicarbonate, $HCO_3/NaHCO_3 = a_1$ by substitution

$$\frac{[H]\,[NaHCO_3]\,a_1}{[H_2CO_3]} = k_1, \quad (7)$$

and

$$[H_2CO_3]\ (\text{or } [CO_2]) = \frac{[H]\,[NaHCO_3]\,a_1}{k_1}. \quad (7\text{A})$$

The constants involved have been determined experimentally for moderate dilutions at the temperature of 25° C. by McCoy, 1903 (12), Seyler and Lloyd, 1917 (17), and Auerbach and Pick, 1912 (2).

The degree of dissociation varies with the concentration, and is given as follows by Seyler and Lloyd :—

Concentration (molar) .	0·05	0·1	0·2	0·3	0·5	1·0
Degree of dissociation .	0·82	0·78	0·73	0·69	0·64	0·52

The *p*H of these solutions is approximately constant over a wide range of concentration. The values can be deduced from the theoretical equations of Prideaux, 1915 (15), which agree with the determinations of Auerbach and Pick.

For solutions of medium dilution Prideaux's equation takes the form,

$$R = \frac{1 + 2\frac{k_2 a_1}{[H] a_2} + \frac{k_w}{[H]\,[NaHCO_3]\,a_3}}{1 + \frac{k_2 a_1}{[H] a_2} + \frac{[H]\,a_1}{k_1}}. \qquad (8)$$

$R = \frac{\text{equivalents of alkali}}{\text{molecules of carbonic acid}}$, which in a fresh bicarbonate solution is equal to unity, and the equation can, therefore, be written for that case as,

$$[H] = \sqrt{\frac{k_1 k_2}{a_2} + \frac{k_1 k_w}{a_1 a_3\,[NaHCO_3]}}. \qquad (8\text{A})$$

In this expression the significance of the symbols is as follows: a_1, a_2 and a_3 represent the degrees of dissociation of sodium bicarbonate, carbonate and hydroxide respectively; k_1 = the "first dissociation constant" of carbonic acid, and k_w the dissociation constant of water. Assuming the following values for the constants, as quoted by Prideaux and others, equation (8A) gives the following figures:—

Table I.

$a_1 = 0{\cdot}8.$ $a_2 = 0{\cdot}8.$ $a_3 = 0{\cdot}9.$

$k_1 = 3{\cdot}04 \times 10^{-7}.$ $k_2 = 6{\cdot}0 \times 10^{-11}.$ $k_w = 0{\cdot}7 \times 10^{-14}.$

$NaHCO_3$ in mols per litre.	pH.	pH experimentally determined by Auerbach and Pick
0·1	8·32	8·35
0·01	8·31	
0·001	8·30	

By substituting these values in equation (7A), the amount of free carbon dioxide in solution can be calculated.

Some of the constants, such as the degrees of dissociation, are only known very roughly, and in consequence the calculated figures are rather loose approximations. Taking the same values for a_1 and k_1 as before, the solutions commonly employed in the next section work out as follows:—

Table II.

$NaHCO_3$ in grammes per cent	CO_2 in cubic centimetres per cent., at N.T.P.
0·02	0·064
0·05	0·16
0·10	0·32
0·20	0·64
0·30	0·96
0·40	1·28
0·60	1·92

Since the degree of dissociation tends to diminish with increasing concentration of bicarbonate, the free carbon dioxide concentration will not rise as here indicated, in direct proportion, but in a continuously diminishing manner. The changes of the degree of dissociation are not known with sufficient accuracy to enable this divergence to be estimated numerically, but over the given range it is probably only slight. In the experimental work the figures given above were taken as representing median values, with an error probably not greater than 10 per cent. As is shown by the experimental results slight differences at the higher carbon dioxide concentrations would not be important.

The Oxygen Titration Technique.

The gas exchanges of the plant during the experiment were estimated in terms of oxygen by means of Winkler's titration. Samples of the solution were drawn off into small flasks of about 140 c.c. capacity, both from the reservoir and from the final pipette. The quantities of oxygen in corresponding lots of solution could then be compared to ascertain the oxygen exchanges of the plant during the time taken for the given amount of liquid to pass over it. With so dilute a solution as N/200 sodium thiosulphate for the final titration, the end point of the reaction with a starch indicator was still very sharp, and consequently extremely small quantities of oxygen could be determined. The delicacy of this method for photosynthetic work is illustrated by a comparison with the baryta titration for carbon dioxide which has been frequently used. As employed by Blackman and Smith with N/10 hydrochloric acid, 1 c.c. of acid is equivalent to about 1·25 c.c of carbon dioxide at N T.P., and one drop of acid (0·02 c.c.) to about 0·025 c.c. In a method described by Blackman, 1895 (5), in which the titration was carried out in an enclosed apparatus under carbon dioxide—free air, N/20 acid and baryta could be used, thereby doubling the sensitiveness of the titration. In the Winkler titration 1 c.c. of N/200 sodium thiosulphate is equivalent to 0·0278 c.c. of oxygen and a single drop (0·02 c.c.) $\equiv$ 0·0005 c.c. Assuming that the assimilatory ratio O_2/CO_2, is approximately 1, this method is about twenty-five times as sensitive as the improved baryta method.

Trial Experiments.

The efficiency of the foregoing technique was tested in three ways. These consisted of (*a*) blank experiments with no material in the plant chamber, (*b*) the effect of time on enclosed specimens of ***Fontinalis***, and (*c*) a determination of the "probable error" of the experimentation.

The first blank experiments led to the discarding of the waxed metal chamber, since there was a measurable absorption of oxygen from the water passed through it, and the substitution of one made of glass. This step was only temporarily successful, as eventually loss of oxygen in passing through the apparatus again appeared, and was traced to the presence of bacteria and a mucilaginous blue-green alga, which adhered to the inner surfaces of the apparatus. This infection only appeared in the parts which the solution passed through after leaving the assimilation chamber, and was no doubt due to the washing off of ephiphytes from the surface of the *Fontinalis*, which afterwards adhered and multiplied, particularly on the sides of the collecting pipettes. The apparatus was, therefore, thoroughly cleaned out with warm water and sterilised with formaldehyde about once a week, and with these precautions blank experiments, which were carried out from time to time, showed no further appreciable loss of oxygen.

To discover any fluctuations due to falling off in photosynthetic activity by the *Fontinalis*, under the conditions imposed by the apparatus, or to any irregularities of the apparatus itself, experiments were performed passing a single solution over the material with constant conditions of temperature and light for several hours. One such experiment may be described in full as typical of most of those which followed.

A number of freshly gathered pieces of *Fontinalis* were put into the plant chamber which was then closed and filled with solution from the reservoir, care being taken not to let any air bubbles remain inside. The chamber was placed on its stand in the thermostat and connected up with the rest of the apparatus. The taps controlling the reservoir and the final pipettes were then opened and the light switched on. The stream of solution at once began to move displacing the tinted alcoholic solution of the pipettes into the measuring cylinder provided. By means of a previous trial, the length of the capillary and the positions of the free water surfaces had been adjusted to give a rate of flow of 400 c c. per hour. At the end of the first hour the three-way tap controlling the pipettes was switched over, and the solution withdrawn from the first pipette for analysis. The result of this titration was compared with one carried out on a sample drawn from the reservoir at the start of the experiment.

Since the capacity of the apparatus was about 400 c.c. the intervening hour represented the time taken by a given particle of liquid to pass through it, and made the comparison more exact than if a control sample had been taken at the same time as the final. Similar readings were taken at the end of each hour, and the results obtained were as follows. The results of the first period had always to be neglected as the steady value had not been reached owing to the initial filling of the chamber with still water. After some experience these first samples were no longer titrated.

Table III.—Trial Experiment with *Fontinalis antipyretica*, April 30, 1925. Solution, 0·01 M. $NaHCO_3$.

Time p.m.	Pipette.	Volume in cylinder in cubic centimetres.	Rate of flow cubic centimetres per hour.	Temperature, ° C.	Oxygen content of solution in thiosulphate units.*		
					Affluent.	Effluent.	Difference
3.2	J	0		18·7	15·19		
4.2	J	410	410			18·35	3·16
	K	0			15 19		
5.2	K	417	417	18·7		18·23	3·04
	J	0			15·20		
6.2	J	398	398	18·8		18 32	3·12
	K	0			15 22		
7.2	K	410	410			18·34	3·12

A similar constancy was shown if the apparatus was darkened and a measurement made of the oxygen absorbed by respiration. In other cases observations were made with a single lot of material under similar conditions on successive days. The *Fontinalis* showed no signs of deterioration under investigation and gave results repeatable after 48 hours. During the interval over night the material was kept in a large bowl of Knop's solution.

The "probable error" of the technique was estimated from an assimilation experiment carried out with light "limiting." The carbon dioxide concentration was not kept constant, and further errors were probably introduced by uncontrollable fluctuations of the light intensity. The error as determined in this experiment would, therefore, be in the nature of a maximum one, since when the carbon dioxide concentration was "limiting" the light fluctuations would be less material, and the concentration could be controlled with greater accuracy than the light. The results of this series, in which six determinations of the assimilation rate were made, were:

"Real Assimilation" = 3·72, 4·07, 3·92, 3·75, 3·72, 3·79.

The "probable error" of the mean was calculated from the usual formula, $P = \frac{2}{3}\sqrt{\frac{\Sigma(v^2)}{n-1}}$, where $\Sigma(v^2)$ = the sum of the squares of the deviations of the

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.c. of N/200 sodium thiosulphate solution. They are left in this form a [illegible] convenient magnitude than the small fractions expressing oxygen quantities. For conversion 1 c.c. N/200 thiosulphate ≡ 0·0278 c.c. O_2 at N.T.P. The quantity of solution taken for analysis was always 100 c.c.

readings from their mean, and n = the number of readings. The value of the "probable error" so determined was ± 0·097, equivalent in this case to about 2·7 per cent. It must, however, be remembered in considering the following experimental results, that differences of less than 3·8 often had to be dealt with, and in those cases the percentage error was naturally greater. The value of 0·097 for the probable error was in agreement with the observed fact, that any alteration showing a greater difference than 0·3 c.c. of titration was almost always repeatable, this being the expected magnitude of the limits of significance (probable error × 3).

EXPERIMENTAL RESULTS.

SECTION I.—*Interaction of the Factors of Carbon Dioxide and Light.*

In this section a number of experiments are described, showing the form of the assimilation curve with low concentrations of carbon dioxide and the modifications introduced by variations of light intensity. The experiments were carried out, save for the necessary small changes, in the manner of the trial described on p. 11. The water plant used for all the experiments in this research was *Fontinalis antipyretica*, gathered from the Cam and kept in a large bowl of Knop's solution in a roof greenhouse until required. The shoots used in an experiment were returned to a bowl of this solution each night while the experiment was in progress. At each change to a different concentration the plant chamber was disconnected from the rest of the apparatus, emptied out and refilled with the fresh solution. The tubes leading up to the chamber were also flushed out with the new solution before the connections were again made and the experiment re-started. The details of the experiments were as follows .

Series 50.—The Relation of Photosynthesis to Carbon Dioxide Concentration at Low Light Intensity.

In this series seven changes of solution were used, varying from carbon dioxide free water to 2·0 per cent. CO_2. The rate of respiration was determined at the start and finish of the series. In calculating the "real assimilation" the observed value of respiration nearest in time to the reading for apparent assimilation has been taken. The difference between the two respiration values is scarcely significant, but a slight falling off in the value of respiration during a prolonged experiment appears to be almost invariable.

Table IV.—Results of Series 50.

30 young 2-inch shoots of *Fontinalis*, collected December 7. Experiment December 8–10. Temperature = 18·3–18·4° C. Light = 15 units.*

Experiment No.	CO_2 in cubic centimetres per cent	Apparent assimilation.	Respiration	Real assimilation.
1	0	−0·95		1·30
2			2·25	
4	0·05	−0·30		1·95
3	0·10	−0·03		2·22
5	0·20	+0·65		2·90
6	0·30	+0·65		2·90
7	0·80	+1·77		3·73
8	2·00	+1·97		3·93
9			1·96	

Series 51.—The Relation of Photosynthesis to Carbon Dioxide Concentration at Low Medium Light Intensity.

The arrangements for this series were similar to those of the last, except that the light intensity was increased to 20 units. Rather more readings were obtained and the form of the curve determined more accurately in consequence. The whole series lasted over three days and a respiration reading was taken each day. As before, there was a very slight falling off in the rate of respiration while the plant was under investigation.

Table V.—Results of Series 51.

Experiment December 14–16 Temperature = 19·0° C. Light = 20 units.

Experiment No.	CO_2 in cubic centimetres per cent	Apparent assimilation	Respiration	Real assimilation.
1	0	−1·10		0·67
2	0		1·77	
4	0·05	−0·79		0·98
3	0·10	−0·40		1·37
5	0·20	+0·25		1·95
9			1·70	
10	0·30	+0·70		2·20
11			1·50	
7	0·40	+1·28		2·98
6	0·80	+1·85		3·55
8	2·00	+2·37		4·07

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* Light intensities are given in arbitrary units; 40 units was the illumination given at the assimilation chamber by a 300-watt lamp at 54 cms. distance.

The curve for these results (fig. 2) shows the same features as that of the previous experiment. There is in both cases a definite "real assimilation" value for zero external carbon dioxide concentration and the remainder of the curve follows a similar path in both experiments. It appears to be of the form of an oblique hyperbola. Plotted logarithmically, the rate of photosynthesis against the log. of the carbon dioxide concentration, the curve takes the general sigmoid form to be expected, but the points are not sufficient to decide the matter accurately. For reasons to be elaborated in connection with later experiments, these results cannot be accepted as final.

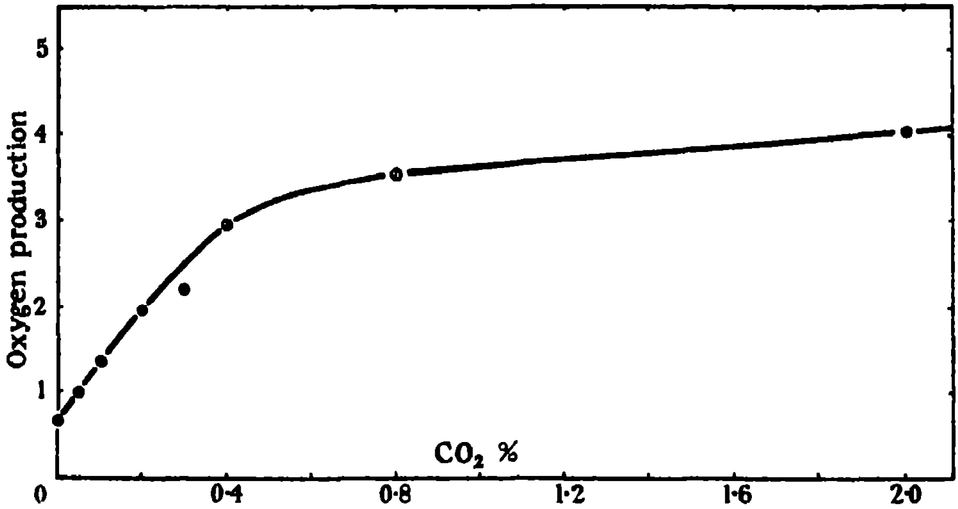

FIG. 2.—Results of Series 51. Curve of assimilation rate in 0–2·0 per cent. carbon dioxide at light intensity 20.

Series 56.—The Rate of Photosynthesis at Low-Medium Light Intensity and rather higher Carbon Dioxide Concentrations.

In both the preceding experiments the assimilation curve was still in a rising phase at the highest concentrations of carbon dioxide used, which was only as much as 2·0 per cent. by volume at N.T.P. The present experiment was designed to investigate a rather higher range of concentrations, and to determine, if possible, whether further additions of carbon dioxide will continue to increase the rate of photosynthesis indefinitely, or whether light or some other controlling factor eventually exercises a "limiting" action, and further addition of carbon dioxide is without effect. The limitations of the method employed debarred the investigation of rapid rates of assimilation, so that a low light intensity was used, which would be likely to become "limiting"* at a fairly low velocity of photosynthesis. Light intensity 20 was found to be weak enough for the purpose, and was used so that the results might be comparable in this respect with those of Series 51. The arrangements were otherwise similar to those of the previous experiments. The rate of respiration unfortunately changed to a considerably greater extent than usual, so that the apparent assimilation curve was much distorted. The real assimilation curve was however, continuous and the readings did not suggest any alteration of photosynthesis with time while the experiment was in progress. The value for 3·0 per cent. carbon dioxide was taken in triplicate, once in the early part of the experiment and twice at the finish, the three values showing good agreement. Owing to the length of time taken to obtain each reading (2 hours) it was necessary to reduce their number as far as possible. In this experiment, therefore, only a few readings were taken in the initial regions of the curve, covered by the previous experiments, attention being here focussed on the higher concentrations.

Table VI.—Results of Series 56.

30 2-inch shoots of *Fontinalis*, collected February 2. Experiment February 3–5. Light = 20 units. Temperature = 20·0° C. Initial Respiration (Experiments 1–4) 1·54. Final Respiration (Experiments 5–10) 0·92.

Experiment No.	CO_2 in cubic centimetres per cent.	Real assimilation.
5	0·0	0·85
1	0·5	2·88
4	1·0	3·66
7	2·0	3·27
3	3·0	3·72
9	3·0	3·75
10	3·0	3·92
2	4·0	3·79
6	5·0	4·07
8	5·0	3·72

From these results it would appear that over the range of 3–5 per cent. and probably from the region of about 2 per cent. CO_2 (see fig. 3) the rate of photosynthesis is practically independent of carbon dioxide concentration. The experimental error is rather high, probably due to uncontrollable fluctuations of light intensity, which here exercise their maximum effect. A second experiment, not set out here, gave similar results with a smaller number of points.

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* The word "limiting" is here used only in the sense of "in the relative minimum," and is not taken to mean the entire absence of influence of all other factors than the one referred to. Unless otherwise stated, it is to be understood in this sense throughout the paper.

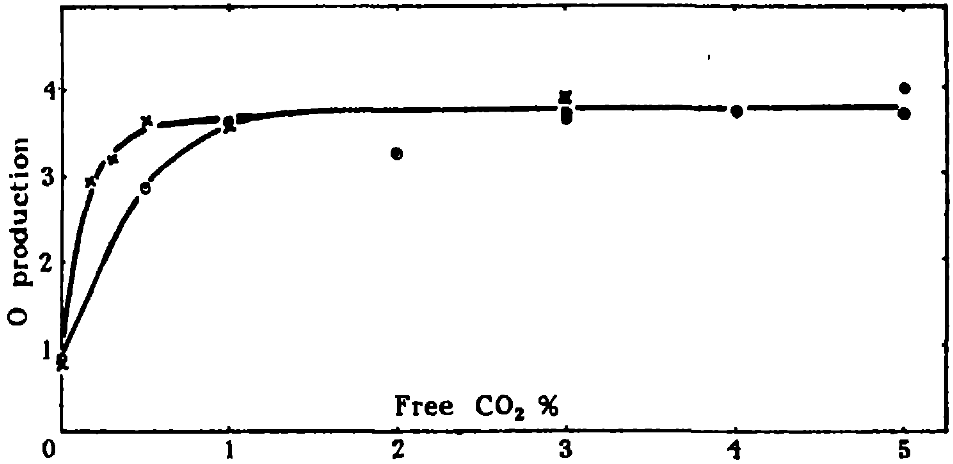

Fig. 3.—Results of Series 56 and 63 plotted together. Rate of assimilation in solutions with 0–5·0 per cent. carbon dioxide moved through the apparatus at 400 and 600 c.c. per hour. ⊙ Series 56; 400 c.c. solution supplied per hour. × Series 63; 600 c.c. of solution supplied per hour.

Series 54 - The Effect of varying both Light Intensity and Carbon Dioxide Concentration.

The form of curve expressing the relation between the rate of photosynthesis and the external concentration of carbon dioxide under a given fixed condition of light intensity has been shown in the foregoing experiments. It remains to discover the effect on such a curve of increases of light intensity at the various concentrations of carbon dioxide employed. The principal difficulty encountered is the amount of time taken to get the rather numerous readings required for such an experiment. If this is allowed to become too long, there is a danger that the material will change while under investigation, and the later readings will consequently not be strictly comparable with the earlier. Under the conditions of experimentation normally applied in this work, it is a matter of experience that no such changes are likely to occur during the first three days (*cf.* Series 56, p. 15); after that the rate of photosynthesis under any given conditions is liable to show a falling off. In general, therefore, experiments were planned to be carried through within this time limit, but the present series had to be an exception, and a depression of photosynthetic activity became apparent in its later stages. The extent of the depression is indicated in the details of the results, so that allowance can be made for it.

Light intensity was varied by raising or lowering the electric lamp to fixed heights above the plant chamber, and measured by means of a large surface thermopile as previously described. The procedure was otherwise the same as before. The results are given in Table VII in the order in which they were obtained.

Table VII.—Results of Series 54.

Fontinalis, collected January 23. Experiment, January 25–29. 30 2-inch shoots. Temperature = 20·0 ± 0·1° C.

Experiment No.	Date, 1926.	CO_2 in cubic centimetres per cent.	Light.	Initial oxygen.	Final oxygen.	Difference.
1	Jan. 25	0	0	18·60	17·25	−1·35*
2	,, 25	0	40	18·85	18·55	−0·30
3	,, 25	0	10	18·75	18·35	−0·40
4	,, 26	0	80	19·25	19·10	−0·15
5	,, 26	2·0	40	19·40	26·90	+7·50
6	,, 26	2·0	10	19·45	20·25	+0·80
7	,, 26	2·0	20	19·45	22·85	+3·40
8	,, 26	0	20	18·95	18·95	+0·00
9	,, 27	0	0	19·20	17·95	−1·25*
10	,, 27	0·5	20	19·31	21·92	+2·61
11	,, 27	0·5	10	19·15	19·61	+0·46
12	,, 27	0·5	40	19·15	23·26	+4·11
13	,, 27	1·0	40	18·92	24·92	+6·00
14	,, 28	1·0	10	19·32	20·00	+0·68
15	,, 28	0·25	20	19·45	20·85	+1·40
16	,, 28	0·25	10	19·55	19·82	+0·27
17	,, 28	0·25	40	19·65	21·40	+1·75
18	,, 28	1·0	20	19·70	22·30	+2·60
19	,, 29	0	0	19·55	18·30	−1·25*
20	,, 29	0·5	20	19·72	21·95	+2·23†
21	,, 29	2·0	20	19·55	22·40	+2·85‡

* Resp. † *Cf.* with 10. ‡ *Cf.* with 7.

Depression of the rate of photosynthesis first becomes apparent in reading 17 as is evident from the graphic representation in fig. 4. Readings 20 and 21 were repetitions of earlier readings, and were taken for the purpose of estimating the extent of the falling off; they are represented in the graph by the dotted line.

The figures obtained in this experiment are sufficient to determine the principal objects aimed at. Although the curves for each individual light intensity may be regarded as composed of two parts, each approximately a straight line over a limited range of carbon dioxide concentrations, the transition from one to another is gradual and not a sudden inflection. It cannot be claimed, however, that the curves for the lower light intensities demonstrate this point, owing to the magnitude of the experimental error, and it is perhaps better illustrated by the curve of Series 51 (fig. 2). In addition the present series shows that the curves for different light intensities are not to be regarded as coincident in their initial stages, but as being separate almost from the start, since the differences shown in these regions are quite significant. The interpretation of these features will be considered in the general discussion of results.

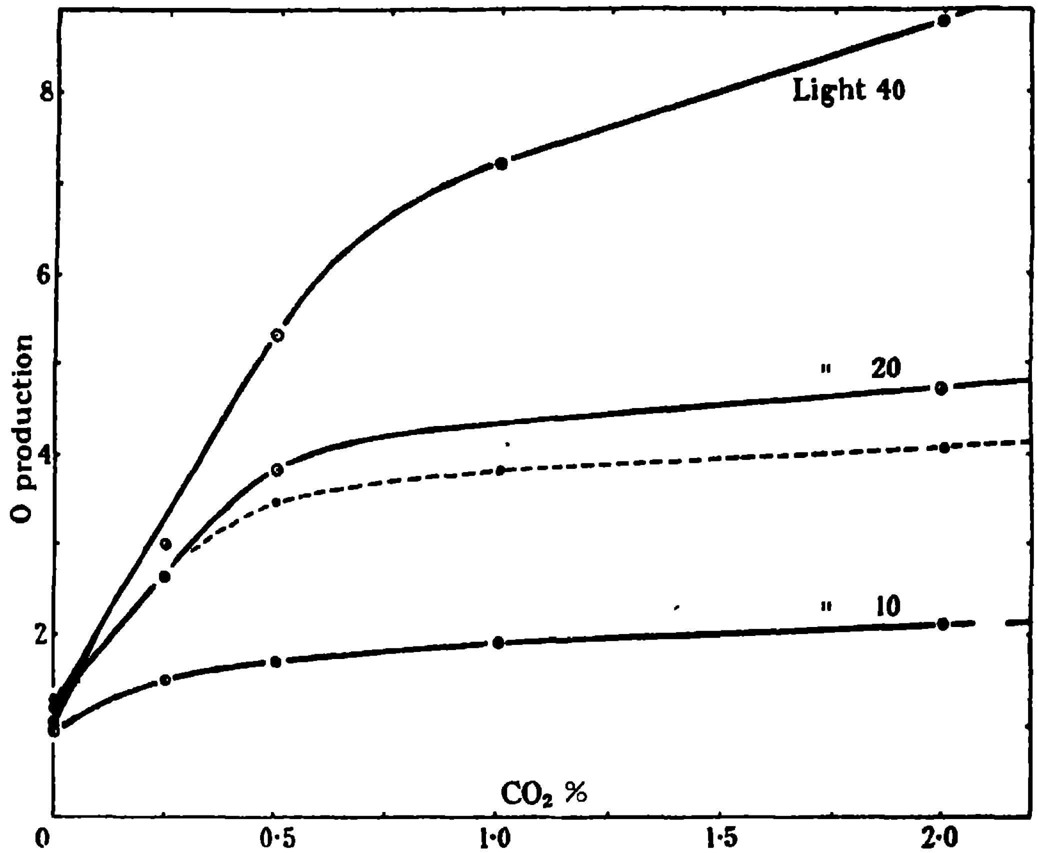

Fig. 4.—Results of Series 54. The rate of assimilation in carbon dioxide concentrations 0–2·0 per cent. and light intensities 10–40.

Section II.—*Effect of Variation in the Method and Rate of Carbon Dioxide Supply.*

A. *The Use of Sodium Bicarbonate as a Source of Carbon Dioxide.*—In the previous section an estimate has been made of the relation between assimilation rate and the concentration of carbon dioxide when the latter is supplied in the form of a solution flowing through the chamber at the rate of 400 c c. per hour. This was followed by an investigation into the effects of supplying the carbon dioxide by different methods. In the first set of experiments now to be described it was supplied by means of dissolved sodium bicarbonate. The series gave anomalous results and is only quoted here as a starting point for those which followed, in which several improvements in treatment were introduced to eliminate any injurious effect of alkaline solutions on the plants. Moderately high concentrations depress the rate of photosynthesis, and the injury may even continue as a permanent after-effect when the plant is removed to other solutions.

In the present experiments none of the solutions used were of sufficient strength to cause noticeable slowing down in the rate of assimilation, but in the latter series it was considered advisable to carry out all the readings with carbon dioxide solutions before putting the material into bicarbonate solutions at all. In this way the chance of any bicarbonate after-effect during the carbon dioxide reading was eliminated. The presence of the bicarbonate appeared also to have an effect on the rate of respiration of the material. In dilute solutions the effect was very small and might even be within the limits of experimental error. In other cases, however, it was considerable, and the only way to be sure about the matter was to take a separate respiration reading in connection with every strength of bicarbonate solution. This was generally done immediately after the measurement of the apparent assimilation, by darkening the plant chamber and continuing to use the same flow of prepared solution. Errors due to change of solution and change of respiration rate with time were thus reduced to a minimum. These precautions were not taken in the first set of experiments, but were in all subsequent ones.

Series 26.—The Effect of supplying Carbon Dioxide by the Dissociation of Sodium Bicarbonate.

In this series the readings were taken in random order both as regards concentration and nature of the solution, to avoid any definite time drift in the curves The respiration was only determined twice, once for each kind of solution. The waxed metal plant chamber, subsequently discarded, was used in this early experiment, so that the measured values of respiration are probably too high, owing to oxidation of the wax. The apparent assimilation values would, however, be correspondingly decreased, so that the net error of the "real assimilation" figures must in any case be very small Later experiments eliminated this source of error also.

A high light intensity, about 80, was used, and as temperature was moderate (about 20° C.) there was no likelihood of light or temperature limiting the reaction and masking any carbon dioxide effects that might occur. To allow of rapid rates being measured, the experimental water was first freed from contained gases by boiling in the usual manner, and then cooled under nitrogen containing only a very small percentage of oxygen. In this way the initial partial pressure of oxygen was reduced to a greater extent than usual, so that larger amounts could be formed by assimilation without saturation of the water causing free bubbles to be given off in the plant chamber. The oxygen content of the water was not reduced too much, lest anaerobic respiration should occur.

Table VIII. -Results of Series 26.

Light about 80 units. Temperature about 20° C.

Solution.		Real assimilation	
CO_2 in cubic centimetres per cent.	$NaHCO_3$ in grammes per cent	In CO_2	In $NaHCO_3$
0	0	0·32	—
0·03	0·01	--	1·87
0·16	0·05	—	5·32
0·32	0·10	2·83	7·13
0·64	0·20	5·15	9·44
1·28	0·40	—	12·66
1·92	0·60	8·26	Bubbles formed
2·76	—	10·19	,,

The difference between the rate of photosynthesis in a solution of carbon dioxide and in a solution of sodium bicarbonate of equal partial pressure appears from these results to be considerable, and is no doubt far greater than could be accounted for by the various sources of error previously considered. Since the experiment was arranged so that carbon dioxide should be the limiting factor, the difference of the observed values suggests a difference of availability of carbon dioxide in the two cases, and the later series of experiments were planned to test this supposition. Alternatively, the bicarbonate divergence might be due to the introduction of sodium ions, increased alkalinity, direct assimilation of HCO_3 ions, differences of osmotic relations, or any combination of such causes.

It may readily be supposed that carbon dioxide supplied in the form of sodium bicarbonate is more available than in the simple solution. In the latter, if there is no movement in the liquid, diffusion shells are gradually set up round the gas-absorbing surfaces, as the carbon dioxide in the immediate vicinity of the plant is used up. The rate of absorption consequently falls off until an equilibrium is attained, which is a function of the rate of diffusion of carbon dioxide in an aqueous medium. In the case of sodium bicarbonate the concentration of the free carbon dioxide is dependent on the dissociation of the salt in solution.

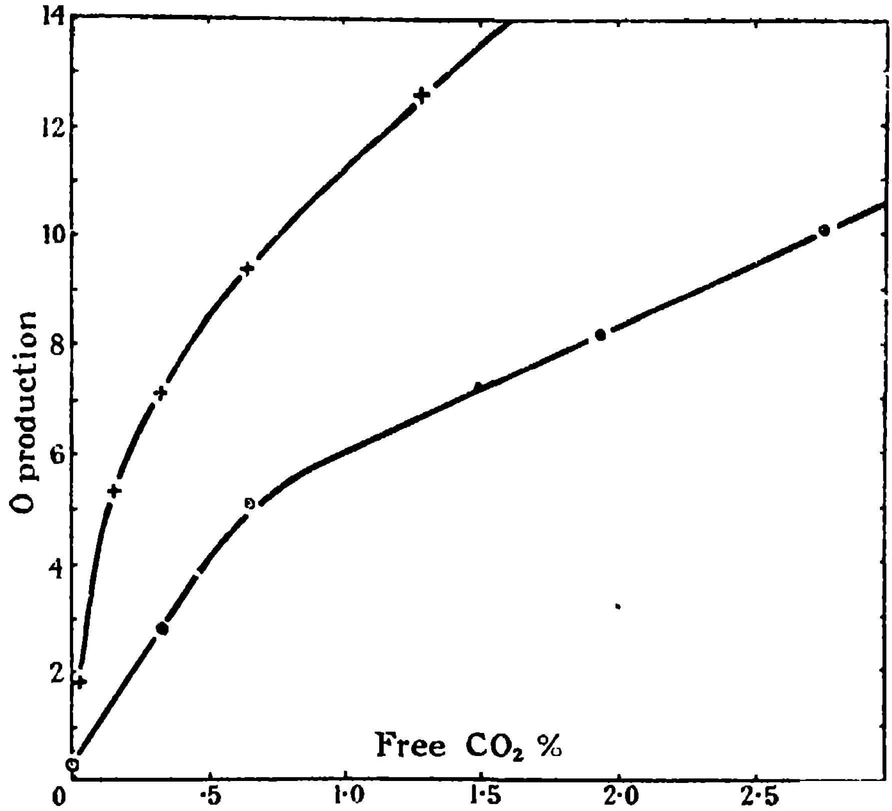

Fig. 5.—Results of Series 26. Rate of assimilation in solutions of carbon dioxide and sodium bicarbonate. ⊙ Oxygen production in carbon dioxide solutions, + in sodium bicarbonate solutions.

As the gas is removed by assimilation, the equilibrium tends to be restored by further dissociation, and it seems to be generally assumed that the rate of this latter process is greatly in excess of assimilation rates. Provided that the change $HCO_3 \rightarrow H_2CO_3$ is actually very rapid the ratio CO_2/HCO_3 in such a solution is of the order of 1/100, so that if the diffusion rates are of comparable magnitude the gradient will be about a hundred times as " steep " as in a simple carbon dioxide solution.

In perfectly still solutions, therefore, sodium bicarbonate might be expected to provide a much more efficient supply of carbon dioxide than the simple solution. If the solutions are stirred, or caused to flow over the absorbing surfaces, as under the conditions of the experiment, the tendency is to break down the diffusion shells by bringing fresh supplies of the gas up to the place of removal, by more rapid means than mere diffusion. To secure uniform distribution of the gas throughout the liquid medium it is theoretically necessary to keep it moving at infinite velocity, so that each particle of gas removed shall be instantly replaced. In practice the problem resolves itself into obtaining such a velocity that no further increase of it will cause any measurable increase in the corresponding rate of removal of carbon dioxide by assimilation.

B. *Alteration of Carbon Dioxide Supply by Changes of Rate of Flow.*—Experiments were carried out to examine the application of the principles just referred to. In the experiments described above, 400 ± 20 c.c. of the solutions were passed through the apparatus per hour. It is not likely that this should be the ideal rate of flow as it only afforded a slow drip from the pipettes. In one solution of the last series, 0·64 c.c. of carbon dioxide at N.T.P. was supplied to the plant in each 100 c.c. of solution. The amount of oxygen produced while this quantity of solution was passing over the plant was 2·41 × 0·0278 = 0·067 c.c. If it is assumed that the assimilation coefficient was equal to unity, and a corresponding amount of carbon dioxide was therefore absorbed, the reduction in the average concentration of the flowing solution was rather over 10 per cent. The reduction in the solution nearest the plant would be much greater. A flow of 400 c.c. per hour cannot, therefore, be entirely efficient in breaking down the carbon dioxide diffusion shells. Since it is necessary to have some appreciable difference in the inflowing and outflowing solutions to allow of measurement, it is obvious that the ideal condition of a constant gas concentration is not attainable; nevertheless a faster rate of flow than the one previously employed might afford a better compromise between the opposing requirements.

Series 68.—*The Effect of increasing the Rate of Supply of the Solution.*

This experiment was an attempt to discover if the rate of assimilation could be increased by increasing the rate of flow of the prepared solutions through the apparatus, and to determine the most suitable rate for obtaining comparable results between carbon dioxide and sodium bicarbonate solutions. Standard conditions were employed of a medium intensity, with carbon dioxide limiting, and the same concentration was passed over the plant material at the various velocities tried. It was necessary to take respiration readings corresponding with every observation of the assimilation. At the end of the readings for each flow the capillary controlling the rate of passage through the apparatus was changed, the apparatus reconnected, and the stream started again by opening the stopcocks.

Table IX.—Results of Series 68.

35 2-inch shoots of *Fontinalis*, collected March 10. Experiment, March 12–13.

Rate of flow in cubic centimetres per hour.	In 15 minutes.		
	Apparent assimilation.	Respiration.	Real assimilation.
430	3 01		
408		1·57	4·58
615	3·90		
600		1 73	5·63
909	3·71		
—		1·69	5·40
1188	0 96		
1200		1·14	2·10

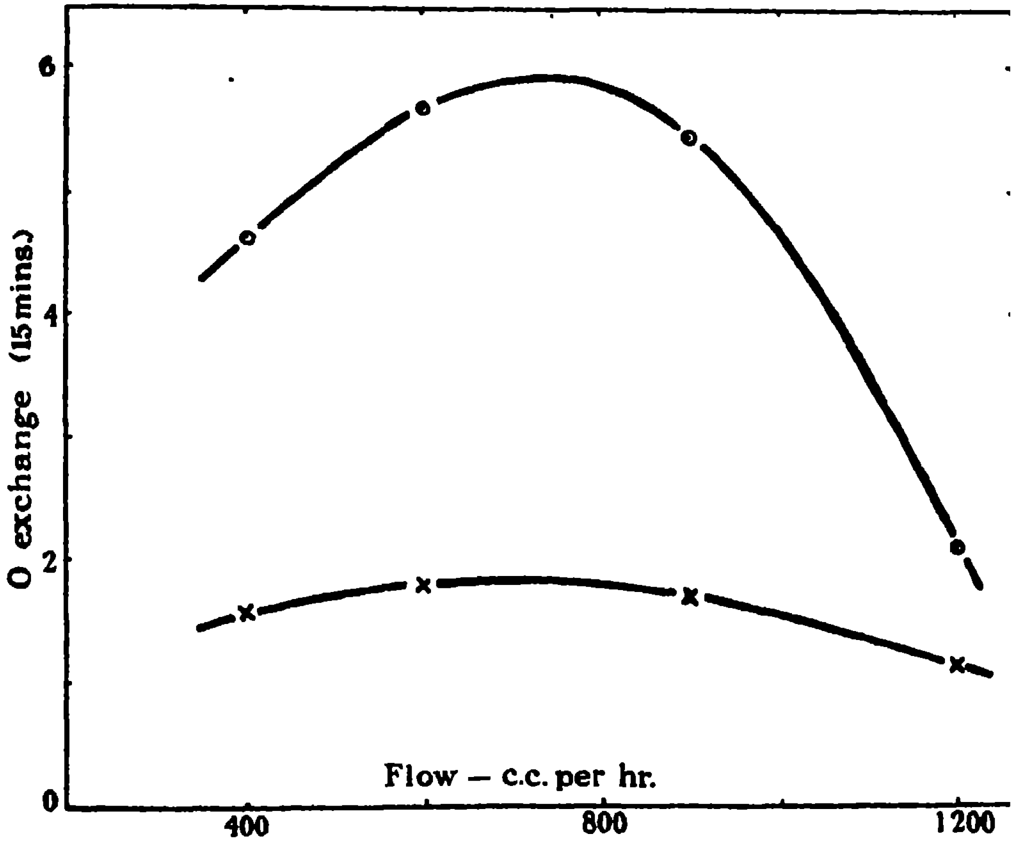

Fig. 6.—Results of Series 68. The relation between oxygen exchange and the rate of flow of a carbon dioxide solution. ⊙ Oxygen produced in assimilation, × oxygen absorbed in respiration.

The samples taken for analysis were 100 c.c. of solution in each case. To make the readings comparable it was necessary to reduce them to a common time value, and 15 minutes was selected to avoid too great a multiplication of the titration figures for the faster rates, with consequent exaggeration of the experimental error. Even so this factor needs to be borne in mind in regard to the values for the rates of 600 and 900 c.c. per hour, where the small observed difference cannot be considered significant. The enormous falling off in the highest rate of flow is difficult to explain on any physiological basis, and whether it is due to complications arising in the apparatus, or to some secondary physiological phenomenon, it is equally to be avoided in investigations of the rate of photosynthesis. The "600" and "900" values show a quite definite increase over the "400" value, and this is of the greatest importance in the connection previously stated. Between the rates of 600 and 900 c.c. per hour, the assimilation may be regarded as practically constant, and at the maximum obtainable with the present apparatus and method for any given solution. It becomes, therefore. of importance to find if the curve relating photosynthesis and carbon dioxide concentration, as determined at these velocities, differs in form from that obtained at 400 c.c. per hour. To decide this a flow of 600 c.c. per hour was used in preference to 900, since although the velocity of photosynthesis was approximately the same at both rates the action of any secondary depressant would necessarily be greater at the latter.

Series 63 and 64.—The Relation of Photosynthesis to Carbon Dioxide Concentration at Low Medium Light Intensity, and Increased Rate of Flow of Solution.

Some preliminary experiments were first done at the increased rate of flow for comparison with Series 51 and 56. There was an interval of about 11 weeks between gathering the material for the first and last series. For this reason only very rough equivalence could be expected between the samples taken, it being a matter of experience that seasonal and other time variations in apparently similar material were considerable, though in the present instance the differences were not very marked. In the experiments with a rate of flow of 400 c.c. per hour 30 shoots of material, each of 2 inches length, were used, and in those with a 600 c.c. per hour rate 45 shoots. The observed oxygen differences in 100 c.c. of solution were thus kept of similar size and a possible source of error due to unequal losses of carbon dioxide from the supplied solutions avoided.

Series 64 was a repetition of Series 63 and confirmed it in every way. The difference between the curves afforded by these two experiments and those for the slower rate of flow is best shown by plotting the results of Series 56 and 63 together as was done in fig. 3. At the higher concentrations, about 1·0 per cent. CO_2, the two curves coincide. They also meet at zero external concentration, but in the intermediate region they diverge considerably. The interpretation of these observations is best reserved until some further experiments have been described.

Since different lots of material were used to obtain the separate curves, their coincidence at the upper and lower extremes is largely fortuitous. On the simple hypothesis to be described such relations between the two curves might be expected, but the evidence as it stands is obviously insufficient to be decisive. The suggestion obtained from the comparison of these two curves was, therefore, followed up by obtaining assimilation values at both rates of flow with a single lot of material.

Series 70.—The Rate of Photosynthesis in Solutions of Carbon Dioxide supplied at 400 and 600 c.c. per hour.

This experiment was designed to give precision to the nature of the difference of form just established, by using only one lot of material throughout, and so rendering all the results strictly comparable. It was carried through in two days to reduce the chances of change in the *Fontinalis*, and to do this the number of readings had to be kept fairly small. A respiration reading was taken each day. On the first day four assimilation readings were taken, with a rate of flow through the apparatus of 400 ± 15 c.c. per hour, and on the second day five assimilation readings with a rate of 600 ± 30 c.c. per hour. Had the two rates been used alternatively it would have been necessary to take respiration readings for both on each day of the experiment, thus introducing two additional periods amounting to several hours. The controlled conditions and the quantity of the material were similar to those used in the previous experiments, but in spite of this the assimilation values were higher than those of the previous series, probably due to seasonal changes in the *Fontinalis*, as this series was carried out in May.

Since it was desirable to use the same material throughout, the observed results for the 400 c.c. per hour rate had to be reduced to the standard form of amounts due to 30 shoots in 15 minutes. Forty-five shoots were used and the observed values reduced to two-thirds in the table. With the 600 c.c. per hour rate the observed amounts were automatically equivalent to the standard.

Table X.—Results of Series 70.

Fontinalis, collected May 2. Experiments, May 3 and 4. Light = 20 units. Temperature = 20·0 ± 0·1° C.

CO_2 in cubic centimetres per cent.	Apparent assimilation. Flow.		Respiration. Flow.		Real assimilation. Flow.	
	400 cubic centimetres per hour.	600 cubic centimetres per hour.	400 cubic centimetres per hour.	600 cubic centimetres per hour.	400 cubic centimetres per hour.	600 cubic centimetres per hour.
0·00	−0·60	−0·10	1·66	1·30	1·06	1·20
0·32	+1·66	+2·65			3·32	3·95
0·64	+2·50	+3·82			4·16	5·12
1·28	+3·56	+4·13			5·22	5·43
2·56	—	+4·20			—	5·50

The difference in the respiration values is almost certainly a time effect and is not connected with the difference in the rates of flow. It is of just about the usual magnitude for the first day's drift, and any change due to the rates of supply would be expected to be in the reverse direction. In Series 68, where respiration readings were taken at both rates on the same day, the difference was negligible, the figures being, at 400 c.c. per hour, 1·05, and at 600 c.c. per hour, 1·15. The difference of respiration in the present experiment causes

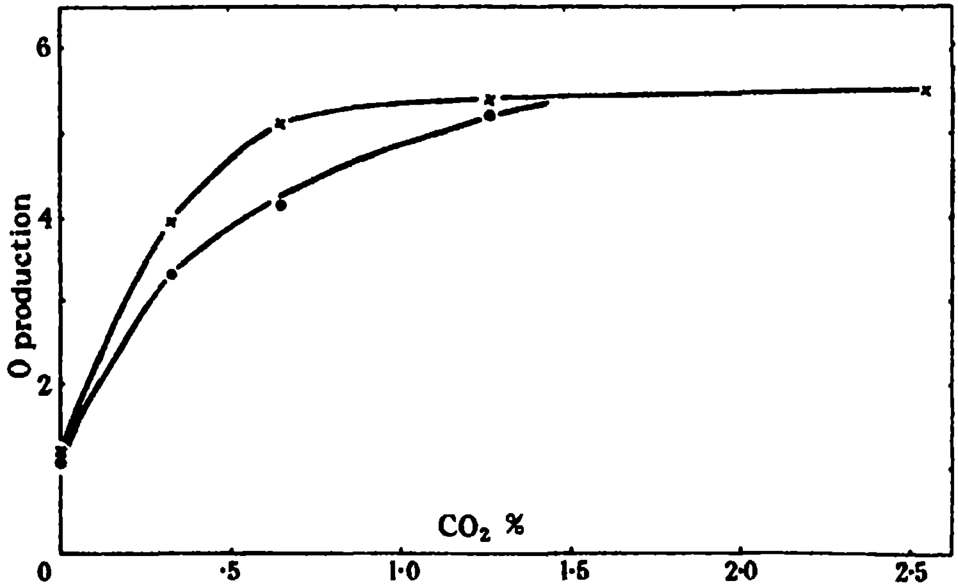

Fig. 7.—Results of Series 70. The relation between assimilation and 0–2·5 per cent. carbon dioxide solutions. ⊙ 400 c.c. of solution supplied per hour, × 600 c.c. of solution supplied per hour.

perceptible distortion in the relation of the two apparent assimilation curves, but should not do so in those for "real assimilation" (fig. 7). These show the features suggested by the comparison of Series 56 and 63 (fig. 3). The two curves again concide at zero external carbon dioxide concentration, are separate over the range up to about 1·3 per cent., and approach again at this point. In this case the absolute values of the two curves are strictly comparable with one another, and it is permissible to draw conclusions based on their relations.

SECTION III.—*Interaction of the Factors of Light and Carbon Dioxide Concentration with Increased Rate of Flow.*

A. ***With Carbon Dioxide Solution as Source of Carbon Dioxide.***—The previous experiments having shown that the form of the curve relating carbon dioxide concentration and assimilation is dependent on the rate at which the carbon dioxide is supplied, it becomes important to determine the series of curves for varying light intensities under the optimal conditions of flow. The most suitable rate of flow having been found, as stated above, to be about 600 c.c. per hour, the experiments in this section were carried out at that rate.

Series 72.—*The Effect of Varying Light Intensity and Carbon Dioxide Concentration.*

With regard to other conditions than the rate of flow this series was virtually a repetition of Series 54, the same light intensities and carbon dioxide concentrations being used. To cut down the number of readings the assumption was made that the rate of assimilation in carbon dioxide—free water was the same at all intensities of light. This is equivalent to saying that in each case the quantity of the respiratory carbon dioxide was low enough to be "limiting," and this is fully borne out by the results of Series 54 and other unpublished experiments. There were no signs of a depression in the assimilatory capacity of the *Fontinalis* while under investigation. The values for "real assimilation" are set out in cross columns on p. 29, and shown graphically in fig. 8 (*a*).

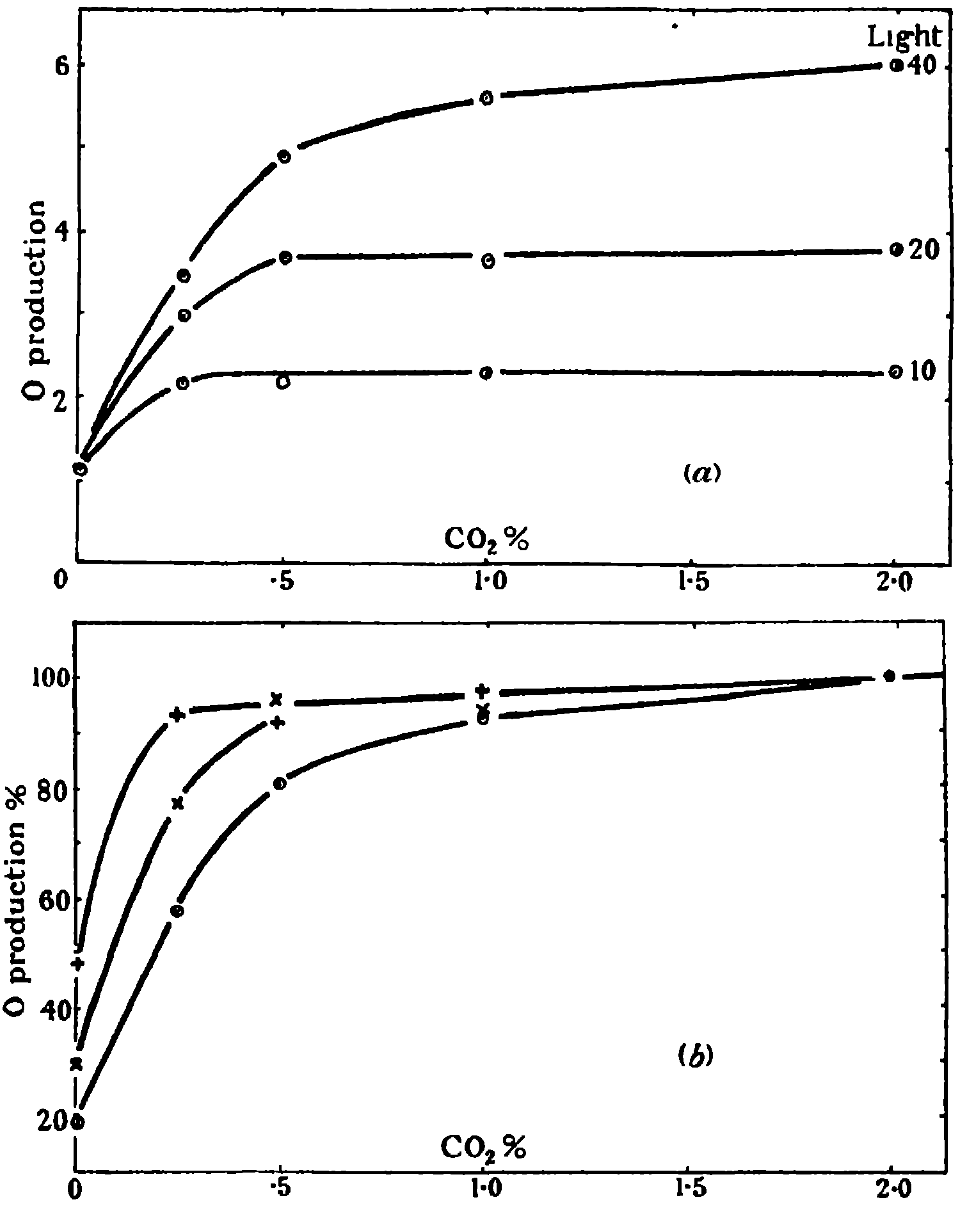

FIG. 8 (*a*.)—Results of Series 72. The rate of assimilation in carbon dioxide concentrations 0–2·0 per cent. and light intensities 10–40. Solutions supplied at 600 c.c. per hour.

FIG. 8 (*b*.)—The same curves plotted to coincide at values for 2·0 per cent. CO$_2$. Light + =10 units, × = 20 units, ⊙ = 40 units. For further explanation see pp. 28 and 29.

The curves in fig. 8 (*a*) show the features that might be expected from a consideration of the previous experiments. They are noticeably more rectangular than those of Series 54 (fig. 4) in agreement with the other results at the faster rate. At zero external concentration of carbon dioxide they are assumed to

Table XI.—Results of Series 72.

45 2-inch shoots of *Fontinalis*, collected May 13. Experiment, May 14–15.

Light.	Carbon dioxide concentration in cubic centimetres per 100 c.c. solution				
	0	0·25	0·50	1·0	2·0
40	1·13	3·48	4·88	5·62	6·05
20		2·98	3·70	3·63	3·85
10		2·20	2·16	2·30	2·35

coincide within the limits of experimental error, but the theoretical conception involved in this will need to be considered further, and they are certainly divergent from a very early stage.

In two curves out of the three, a "light limited" condition is reached, but at the third and highest light intensity it is not. The progress of the upper curve towards the horizontal is clearly a gradual one. In contrast with the present series the two curves for the weaker lights in Series 54 were still in a rising phase at the maximum carbon dioxide concentration used. This difference is in agreement with the results of Series 70, where the two conditions were studied on the same plant material.

B. *With Bicarbonate as Source of Carbon Dioxide.*—As a result of the last set of experiments one is in a position to reconsider the bicarbonate results of Series 26. It is evident from the foregoing section that the form of the assimilation-carbon dioxide concentration curve can be altered by increasing the rate at which the carbon dioxide solution is supplied, and it remains to be seen whether the alteration due to rate of flow is of the same kind as that caused by supplying the carbon dioxide in the form of bicarbonate. In Series 26 (fig. 5) the two curves were rapidly separating at the highest values obtained. There, however, light was very greatly in excess, and neither curve had reached its region of inflection towards the horizontal. It might be that had it been possible to follow out the curves into higher concentrations they would have approached again in a similar manner to those of Series 70. If the two effects are of a similar nature, the curves for the increased rate of flow should approximate to the bicarbonate form, and perhaps even coincide.

Series 63, *b, and* 64, *b.*

When carrying out Series 63 and 64 a few readings were also taken with solutions of sodium bicarbonate, passed through the apparatus at the same rate of 600 c.c. per hour. Only a few could be obtained on account of the time that the material was under investigation. In the following table the values are given with the corresponding carbon dioxide values.

Table XII.

Light = 20 units. Temperature = 20·0 ± 0·1° C. Rate of flow = 600 ± 30 c.c. per hour.

Series No.	$NaHCO_3$ grammes per cent.	CO_2 in cubic centimetres per cent	Apparent assimilation.		Respiration		Real assimilation.	
			$NaHCO_3$.	CO_2.	$NaHCO_3$.	CO_2.	$NaHCO_3$.	CO_2.
64	0·05	0·16	1·65	1·75	1·04	1·20	2·69	2·95
64	0·10	0·32	2·40	2·07	1·20	1·56	3·60	3·63
63	0·156	0·50	2·37	2·45	1·30	1·22	3·67	3·67

The differences in the respiration figures are here again due to time drift. The differences in real assimilation are in each case reduced within the significant limits of experimentation, though the first reading is near the border line. All three readings are below 1·0 per cent. carbon dioxide concentration, and therefore in the region of divergence of the 400 and 600 c.c. per hour curves for carbon dioxide solutions for the given light intensity.

As far as these results go, therefore, they fulfil the expectation that pure carbon dioxide solutions, supplied at a fast enough rate, will have the same assimilatory effect as bicarbonate solutions with an equivalent carbon dioxide concentration. The following series supplied further confirmation.

Series 65.—*The Rate of Assimilation in Solutions of Carbon Dioxide and Sodium Bicarbonate with equal Partial Pressures of Free Carbon Dioxide.*

This series was arranged somewhat differently. The principal object was to compare the rates of assimilation in corresponding pairs of solutions. It was also important to know in what part of the carbon dioxide-assimilation curve the selected concentrations lay. To be sure of this the conditions most frequently used previously were again employed, viz., a light intensity 20 and a temperature of 20·0° C. Instead of using the same plant material throughout fresh material was taken for each pair of solutions. Forty-five shoots, each 2 inches long, were nipped off for each experiment, only well-grown pieces of the largest size being taken. It was hoped in this way to get approximately equal samples, since no seasonal changes had to be considered, and in the result at least a rough comparability seemed to hold, and strict equivalence was kept between each pair of solutions tried. It seemed better to arrange the experiment in this way rather than to use the same material throughout, to avoid any possible after-effect of the sodium bicarbonate, and at the same time to be able to use each pair of solutions consecutively. In all other respects the experiment was carried out in the usual manner.

Table XIII.—Results of Series 65.

Fontinalis, collected March 1. Experiment, March 1–5. Light = 20 units. Temperature = 20·0 ± 0·1° C. Rate of flow = 600 ± 30 c.c. per hour.

$NaHCO_3$ grammes per cent.	CO_2 in cubic centimetres per cent.	Apparent assimilation		Respiration.		Real assimilation.	
		In $NaHCO_3$	In CO_2	In $NaHCO_3$	In CO_2	In $NaHCO_3$	In CO_2
0·05	0·16	1·18	0·62	0·99	1·24	2·17	1·86
0·08	0·24	1·60	1·22	0·97	1·28	2·57	2·50
0·10	0·32	1·70	0·96	1·30	1·82	3·00	2·78
0·20	0·64	1·75	1·98	1·44	1·16	3·19	3·14

In these results (see fig. 9, lower curves), as in those of the previous table, the differences of real assimilation in each pair of solutions is small. With the exception of the first they are probably not significant and even that is rather doubtful. It is noticeable, however, that the bicarbonate value is always greater than the carbon dioxide value, and it is just possible that at the lower concentrations this difference is real.

Under the conditions of these two sets of data it seems safe to say that any difference existing between the rates of assimilation in carbon dioxide and equivalent sodium bicarbonate solutions is almost negligibly small, over the range of concentrations tried. In the following series the conditions were changed by using a higher light intensity.

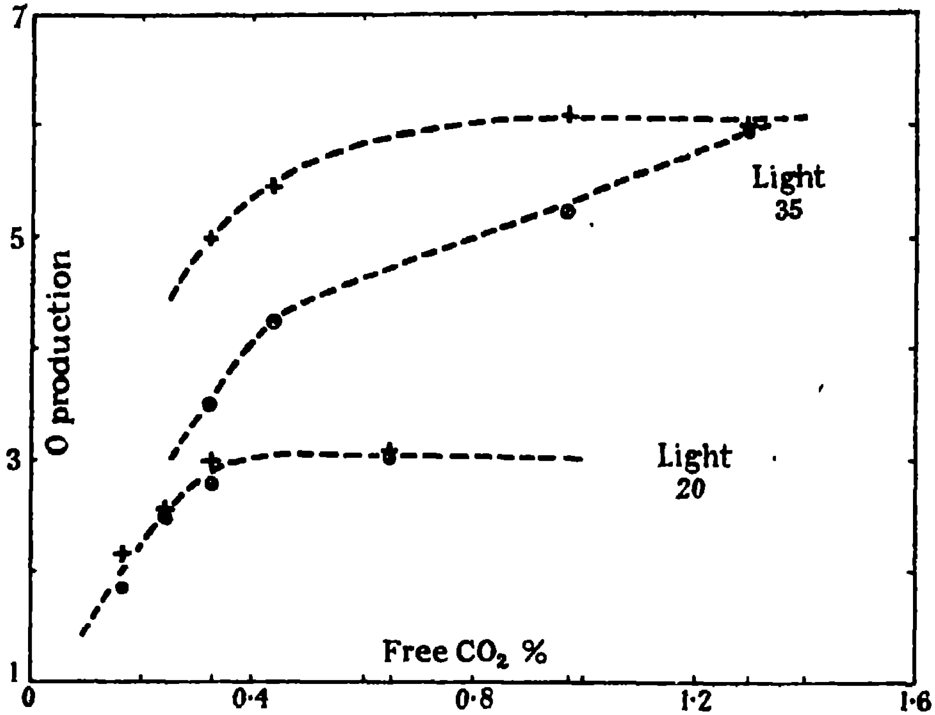

Fig. 9.—Synthetic curves for Series 65 and 69. The relation between assimilation and free carbon dioxide concentration in solutions of carbon dioxide and sodium bicarbonate at lights 20 and 35. ⊙ oxygen production in CO_2 solutions, + in sodium bicarbonate solutions.

Series 69.—*The Rate of Assimilation in Solutions of Carbon Dioxide and Sodium Bicarbonate of equal Partial Pressure of Carbon Dioxide at Medium Light Intensity.*

The *Fontinalis* for this set of experiments was collected on March 15, a fortnight after that for Series 65.

Rough equivalence was secured between the separate lots, and strict equivalence for each pair of solutions in the same manner as before.

Table XIV.—Results of Series 69.

Fontinalis collected March 15. Experiments, March 16–20. Light = 35 units. Temperature = 20·0 ± 0·1° C. Rate of flow = 600 ± 30 c.cs. per hour.

$NaHCO_3$ grammes per cent.	CO_2 in cubic centimetres per cent.	Apparent Assimilation.		Respiration.		Real Assimilation.	
		$NaHCO_3$.	CO_2.	$NaHCO_3$.	CO_2.	$NaHCO_3$.	CO_2
0·100	0·32	3·63	2·20	1·35	1·31	4·98	3·51
0·134	0·43	3·96	2·52	1·48	1·73	5·44	4·25
0·300	0·96	4·62	4·23	1·45	1·00	6·07	5·23
0·400	1·28	4·38	4·72	1·60	1·22	5·98	5·94

By comparison with the previous figures the differences of real assimilation are here quite striking, and, with the exception of the last, well above the limits of experimental error. These results are brought together graphically in the upper curves of fig. 9, which indicates the positions of the various concentrations on their respective assimilation curves. It is noticeable that the divergences and final meeting of the curves for light 35 are of the same nature as in the carbon dioxide curves at light 20 described previously under Series 70.

In most experimental work where bicarbonates have been used as a source of carbon dioxide, the solutions have been used in the still condition, as in the experiments of Harder, Wilmott, etc. It is of great practical interest, therefore, to know whether the movement of the liquid has the same sort of effect in bicarbonate solutions as it has in the case of carbon dioxide. At various times when observations of assimilation in bicarbonate solutions were taken, a reading was also obtained, with the same lot of solution and material, with still liquid in the plant chamber. This was done by disconnecting the chamber from the rest of the apparatus and removing the central inlet tube. The contained liquid was emptied out, and the flask refilled with fresh solution which had previously been brought to the temperature of the bath. The vessel was closed with a rubber stopper coated on the inner surface with a thin layer of paraffin wax, care being taken not to include any air bubbles. The chamber was then immediately immersed in the thermostat in the position it normally occupied. At the end of half an hour, or in some cases 20 minutes, it was removed and the water poured off into a flask and analysed for oxygen in the usual way. A sample of the solution was also analysed initially. These readings, whether for apparent assimilation or for respiration, were always taken either immediately before, or in the majority of cases immediately after, the normally obtained reading with a moving stream of solution with which they were to be compared. As far as possible differences other than the movement of the water were by these means avoided. In the nature of the case it was impossible to eliminate certain others which formed possible sources of error. Among these are the risk of local alkalinity and of local supersaturation with oxygen. The duration of the experiments was kept as brief as possible to minimise these risks, but this brevity in itself tends also to be a source of error.

All the results obtained of this nature are collected together in the following list. The assimilation and respiration quantities are given in the standard form of amounts due to 30 shoots in 15 minutes. This does not affect the comparison of the results, which are only equivalent horizontally in the table.

Table XV.

Light = 20 units. Temperature = 20 ± 0·1° C.

Series No.	$NaHCO_3$ grammes per cent.	Apparent Assimilation.		Respiration.			Real Assimilation.		
		Moving.	Still.	Moving.	Still.	Difference.	Moving.	Still.	Difference.
57	0·02	1·00	1·15	1·05	0·71	+0·34	2·05	1·86	+0·19
65	0·05	1·18	1·47	0·99	1·06	−0·07	2·17	2·53	−0·36
66	0·05	1·10	1·43	1·20	1·12	+0·08	2·30	2·55	−0·25
65	0·08	1·60	1·41	0·97	0·73	+0·24	2·57	2·14	+0·43
60	0·10	1·75	2·50	1·45	0·97	+0·48	3·20	3·47	−0·27
67	0·10	3·41	3·76	1·20	0·79	+0·41	4·61	4·55	+0·06
71	0·10	3·62	3·15	1·98	2·40	−0·42	5·60	5·55	+0·05
58	0·20	2·85	2·77	1·50	0·68	+0·82	4·35	3·45	+0·90
65	0·20	1·75	1·76	1·44	0·59	+0·85	3·19	2·35	+0·84
*73	0·20	5·74	5·66	1·13	0·98	+0·15	6·87	6·64	+0·23
65	0·30	1·65	2·00	1·18	0·74	+0·44	2·83	2·74	+0·09
71	0·30	3·70	4·12	1·84	1·68	+0·16	5·54	5·80	+0·26

* In this reading, light = 40 units.

These values cannot be said to show any great regularity, but in view of the various difficulties already mentioned that could hardly be expected. With the exception of two of the values for 0·2 per cent. sodium bicarbonate the agreement is moderately good. There are ten values in addition to the two already mentioned, representing concentrations of bicarbonate from 0·02–0·3 per cent., which include the whole region where a divergence between the moving and still conditions, if any, might be expected to arise, with the light used. If the differences shown by the two aberrant values have a real physiological significance it should, according to the experience of former results in Series 65, be possible to increase them by speeding up the rate of photosynthesis with an increase of other controlling conditions. During the following series, therefore, the opportunity was taken to obtain a comparison between the effects of moving and still 0·20 per cent. sodium bicarbonate solutions with a doubled light intensity. As shown in the previous table the difference in this case was not significant, and it was accordingly concluded that no particular meaning was to be attached to the two abnormal differences. Of the other values three showed assimilation greater in the still solution, and seven showed it less.

It may, therefore, be said that within rather wide limits of error the rate of photosynthesis in moving and still sodium bicarbonate solutions of the same strength does not differ.

Series 73.—*The Rate of Photosynthesis in Solutions of Sodium Bicarbonate Flowing at Different Rates.*

Comparisons between two rates of flow could be made with greater accuracy than between still and moving solutions with the available method. Results of this nature might, therefore, give clearer indications of the effect on photosynthesis of movement in these solutions than the experiments just described. Since any effect would almost certainly be smaller between two moving solutions, than between the faster movement and stillness, it was decided to use the maximum velocities of assimilation, and consequently the greatest opportunity for differences, that were possible with the technique. For this purpose the light intensity was doubled as compared with the last series, that strength giving about the fastest assimilation velocities that could be measured, when used in conjunction with the strongest solutions which it was desired to examine. The rates of flow were 400 and 600 c.cs. per hour as previously, the reasons for using these particular rates being described under Series 68. Fresh material was used for each comparison, and concentrations of bicarbonate were taken such that carbon dioxide was certain to be the "limiting" condition. Respiration readings were taken immediately after each apparent assimilation value to obtain the best possible correction for real assimilation in each case The results obtained were as follows.

Table XVI.

Light = 40 units. Temperature = 20·0 ± 0·1° C.

Date, 1926.	$NaHCO_3$ grammes per cent.	CO_2 in cubic centimetres per cent.	Apparent Assimilation.		Respiration.			Real Assimilation.		
			600 cubic centimetres per hour.	400 cubic centimetres per hour.	600 cubic centimetres per hour.	400 cubic centimetres per hour.	Difference.	600 cubic centimetres per hour.	400 cubic centimetres per hour.	Difference.
June 1	0·02	0·06	0·75	1·02	1·86	1·40	+0·46	2·61	2·42	+0·19
June 2	0·05	0·16	3·17	3·43	1·57	1·44	+0·13	4·74	4·87	−0·13
May 19	0·10	0·32	4·23	4·01	1·57	1·69	−0·12	5·66	5·70	−0·04
May 17	0·20	0·64	5·40	5·74	1·55	1·13	+0·42	6·95	6·87	+0·08

In these experiments the differences of real assimilation are so small as to be quite negligible, and the results consequently confirm those given in the previous table. The distinction in this respect between carbon dioxide and sodium bicarbonate solutions is clear and uniform.

Discussion.

It is nowadays customary to regard photosynthesis as a complex of reactions falling under the headings of (1) a diffusion phase, (2) a photochemical phase, and (3) a chemical, or " dark," phase. It is proposed in this section to consider the preceding experimental results principally in relation to the first of these stages, where they are chiefly of significance. In dealing with water plants two stages in diffusion have to be considered, the movement of carbon dioxide in the aqueous medium up to the plant surface, and the diffusion thence to the surface of the chloroplasts. The diffusion of carbon dioxide in water is slow and consequently this external effect cannot be neglected, as it may be in the case of land plants where air currents and the relative rapidity of diffusion make its effects inappreciable. The path from the outside surface of the plant up to the chloroplast is normally very short in aquatics whose assimilating organs are usually thin, with the result that the total resistance to diffusion in this phase tends to be low.

In the gaseous exchanges of plants only the external concentration of gases is open to direct control by the experimenter. The diffusion factors affecting the concentration at the surface of the chloroplast are the resistance offered to the passage of the gas from outside, and the difference between the concentrations at the two ends of the path of diffusion. It is of the greatest interest in connection with assimilation problems to fix the relation between the chloroplast-surface concentration and the rate of the process, and this can only be arrived at indirectly by a consideration of the gaseous diffusion and its effects on the system.

A theoretical scheme has already been worked out by Maskell, 1925 (10), for the analogous case of land plants. Here the stages of diffusion are the passages leading up to the assimilating cells, stomata and intercellular spaces, and the passage from the cell surface to the chloroplast. The second of these is practically identical with the corresponding stage in water plants. The resistance of the stomatal phase is variable in its magnitude and, when high, introduces problems similar physiologically to those of diffusion through the water layers surrounding aquatics. Maskell was able to show that variations in the diffusion resistances should have certain definite effects on the curves expressing the relation between *external* carbon dioxide concentration supplied and the rate of photosynthesis.

For apparent assimilation he made the following evaluation, in terms of concentrations and resistances of the processes involved,

$$AA = \frac{C - C_s}{r_1 + r_2} = \frac{C_s}{\dfrac{C_s + k_L}{L}},$$

where $C =$ the external concentration of carbon dioxide, $C_s =$ concentration of carbon dioxide at the chloroplast surface, $L =$ light intensity, $r_1 + r_2 =$ resistances of the two diffusion phases, and $k_L =$ dissociation coefficient of the compound formed by some photochemical product and carbon dioxide (Warburg).

Substituting arbitrary values in these equations he showed that the form of the AA/C curve took on varying shapes for varying values of $r_1 + r_2$, in such a way that the smaller the value of $r_1 + r_2$, under otherwise constant conditions, the more rectangular was the AA/C curve. It will be seen that the changes of form in these theoretical curves are similar to those which occur in the experimental results given above. Increasing the flow of the carbon dioxide solutions in these experiments had an analogous effect to reduction of the diffusion resistances in Maskell's deduced curves.

It is clear, moreover, that the effect of the movement of the solution is to reduce the diffusion resistance in the layers surrounding the plant, since diffusion shells are thereby broken down and high concentrations brought much nearer to the absorbing surfaces. The faster the flow the nearer will the concentrated layers be brought to the surface of the plant, and the steeper will the diffusion gradient become. The smaller the diffusion resistances, to whatever cause they may be due, the nearer one approaches to the ideal condition of a chloroplast surface concentration equal to that outside, and consequently experimentally controllable. The results of a series which is the nearest approximation to this condition that could be obtained, have been described on p. 27. The curves in that experiment, although more rectangular than those obtained with higher diffusion resistances, do not show a sudden bend in their transition regions, which might, perhaps, be thought to be the limiting case.

The action of bicarbonate solutions is of great interest in this connection. Increasing the rate of flow of these solutions had no effect on the rate of photosynthesis, a result in sharp contrast with the state of things in solutions containing only carbon dioxide. In low light intensities, and consequent slow rates of assimilation, the bicarbonates gave rise to a production of oxygen just about equal to the highest obtainable by movement of the carbon dioxide solutions; in high light intensities they caused a rate which could not be equalled by a rapid flow of a corresponding solution of the gas. It appears from these results that the "buffer action" of the bicarbonate is more effective in maintaining the close-up carbon dioxide supply than any practicable movement of the solutions, and may reduce diffusion resistance to a very low value. Since under favourable conditions a pure carbon dioxide solution could bring about the same rate of reaction as a bicarbonate solution the experiments provide strong evidence that only the free carbon dioxide is normally used in assimilation.

The fact that the assimilation-concentration curve is constant in its form in this class of solutions, and corresponds with the best curves obtainable by direct measurements with carbon dioxide, suggests that it is something very near the desired curve expressing the relation of the velocity of the process to the concentration at the chloroplast surface. There does not, for example, appear to be any reason to suppose that still faster movement of the solutions would eventually give a curve with a sharp bend. When it is attempted, however, to define these curves at all closely considerable difficulty arises. Not only is it difficult to obtain a large enough number of points for sharp definition, but the respiratory carbon dioxide effect obscures the lower regions.

The concentrations shown on the abscissæ should really be written as $[CO_2] + x$, x being pictured as an additional concentration of carbon dioxide due to local respiration, and at the lower concentrations used x seems to have been of comparable magnitude with the supplied $[CO_2]$. The form of the curve makes extrapolation for a value of x impossible, and even if this were not the case diffusion relations would make the value so obtained of very doubtful meaning. It is possible that this particular effect would not appear when low concentrations of a bicarbonate are employed, and the results of Warburg with *Chlorella* lend colour to this view.

A further difficulty lies in the correction to be made to convert apparent into real assimilation. It has been shown by Meyer and Deleano (12), Spoehr and McGee, 1923 (18), and by Briggs (8) working on the data of Warburg and Uyesugi, 1924 (21), that there are reasons for supposing that the rate of respiration during assimilation is higher than when it is measured in the dark. If this is so the added correction is too small and all the values of " real assimilation " shown are too low. From the practical point of view it may be pointed out, however, that some unpublished experiments by the present author failed to show any *measurable* effect of this kind in the particular species used for this work, viz., *Fontinalis antipyretica*. The experiments were not, however, of a conclusive kind.

The curve of fig. 2 is almost a straight line in its earlier portions, but this linearity disappears almost entirely in curves obtained with a faster rate of flow of solution, tending to reappear, however, in the corresponding curves for higher light intensities. This is illustrated by replotting the curves of Series 72 (see figs. 8A and 8B) to make them coincide at a point, when a progressive falling away appears in the lower regions of the curves for higher light intensities.

It is probable, therefore, that the linearity that various workers have found at low concentrations* is due to the conditions of diffusion obtaining in their experiments rather than to the internal stages of photosynthesis. While it cannot be definitely stated from the foregoing experimental results that the relation between surface concentration and assimilation shows a falling-off right from the start, there is, on the other hand, no evidence that strict proportionality holds over any range of concentrations. By some writers assimilation curves more or less of this type have been called logarithmic, but there are no real grounds for selecting this rather than other types of " smooth " curve, and before such discriminations can be made very much more detailed results are required than any at present in existence.

The most acceptable idea at present of the relation between the successive stages of photosynthesis is to regard them as a series of linked reactions, each member of the series being reversible. Willstätter (22) and Warburg (20) have put forward views on the nature of these stages. It is possible to insert velocity constants in general terms for the various reactions they have suggested and this has been done by Briggs (8). From a mathematical treatment he concludes that, on the above assumption, the curve expressing the relationship between assimilation rate and the concentration of carbon dioxide at the chloroplast surface should take the form of a hyperbola. The experimental curves of the foregoing sections appear to be of this kind, but the reservation must still be made that the points on them are insufficient for fine discriminations. The cumulative evidence of this and other work does, however, appear to be in favour of a hypothesis such as that mentioned above, the minor differences in observed results being in all probability due to dissimilar diffusion conditions in the various methods of experiment.

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* *Cf.* Stiles (19), " . . . most workers are agreed that in lower concentrations of carbon dioxide the rate of photosynthesis . . . is approximately proportional to the carbon dioxide concentration " ; also Benecke-Jost (4).

Summary.

1. A reinvestigation of the relation between carbon dioxide concentration and the rate of photosynthesis in water plants is attempted, with special reference to the conditions of supply of the carbon dioxide.

2. An apparatus is described, with which a considerable range of conditions can be investigated.

3. The form of the relation between assimilation, carbon dioxide concentration and light intensity is determined with a flow of solution of 400 c.cs. per hour.

4. The effect of modifications in the carbon dioxide supply is investigated by the use of sodium bicarbonate solutions and different rates of flow.

5. At 400 c.cs. per hour sodium bicarbonate is found to give rise to a higher rate of assimilation than a pure solution of carbon dioxide of an equal partial pressure, when no other factor is limiting.

6. At 600 c.cs. per hour, with low light intensity, and consequent slow assimilation, the two solutions give the same assimilation. It would appear, therefore, that in bicarbonate solutions only the free carbon dioxide is available for assimilation. At a higher light intensity the bicarbonate again gives rise to a faster rate than the carbon dioxide solution.

7. The curves relating both carbon dioxide concentration and light intensity to assimilation are redetermined at an optimal rate of flow (600 c.cs. per hour), and it is concluded that they are a series of smooth curves of hyperbolic form.

8. Increases in the rate of flow of sodium bicarbonate solutions are found to be without effect on the velocity of assimilation. In dilute solutions, where

the alkalinity is not great enough to be injurious, these solutions appear to be a satisfactory medium for determining assimilation rates.

9. The nature of the curves obtained is discussed in relation to the diffusion phase of photosynthesis.

I wish to express my gratitude to Dr. W. Stiles for suggesting this investigation, to Dr. F. F. Blackman under whose direction the bulk of it was carried out, and to Mr. G. E. Briggs for stimulating criticism and advice.

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The Yearly Variations in Plague in India in Relation to Climate: Forecasting Epidemics.

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[Plates 1—4.]

It has long been known that plague in India, and elsewhere, has a definite seasonal incidence year after year in the same area, although the months of maximum prevalence vary in different provinces. E. H. Hankin as early as 1905 pointed out the disappearance of dog fleas in the hot weather in Agra, and he suggested that a similar decline in the rat fleas might account for the great fall in plague in the United Provinces of India during the hot season. The extensive and valuable Reports on Plague Investigations in India, published in the *Journal of Hygiene* from 1906 to 1917, contain numerous data on the subject, and they bring out the unfavourable effects of high temperature and low humidity on the duration of life of rat fleas separated from their hosts, and on their rate of multiplication, and they thus accounted for the seasonal incidence of the disease in different areas of India. Further, the work of Bacot in England and of unnamed workers in Poona on the bionomics of fleas showed that relative humidities below 50° to 55° F. and high temperatures are unfavourable in England, and that in Poona, on the Deccan plateau of Western India, the life of fleas was five times as great in August, with an 8 a.m. relative humidity of 80 per cent., as with one of 45 per cent. in April and early May, when plague is at a minimum in that area, where the temperatures are moderate and play much less part than humidity in controlling the plague season. Moreover, studies of the seasonal incidence of plague in different parts of India by the same investigators, working under the Advisory Committee nominated by the India Office, the Royal Society and the Lister Institute, showed that a temperature of 85° F. caused a more rapid disappearance of plague bacilli from the stomach of fleas, with reduced power of infecting animals, as compared with a temperature of 70° F., and with the low mean temperature of 50° F. a large number of the infected rats died before plague bacilli appeared in their blood, so that their fleas had no chance of becoming infected. Rat fleas were also more numerous during the height of the plague season, so that climatic conditions clearly acted by reducing the number of infected fleas.

The incidence of plague in different areas of India was also reported on by R. St. John Brooks (1) in relation to temperature and saturation deficiency, the latter term indicating " the difference between the actual tension of aqueous vapour present in the atmosphere at the temperature in question and the tension of aqueous vapour that would be present in a saturated atmosphere at the same temperature." It is thus a measure of the drying capacity of the air, and Brooks concluded that a degree of dryness indicated by a saturation deficiency of 0·300 inch (measured in terms of mercury) suffices to prevent plague continuing to be epidemic, if the mean temperature is also over 80° F., and that a high saturation deficiency rapidly brings an epidemic to an end even with a temperature below 80° F., but an epidemic may commence and increase with a temperature over 80° F., if the saturation deficiency is below 0·300. Moreover, in certain places, including Java and Mauritius, with constantly favourable temperature and saturation deficiency, the disease may be prevalent indifferently at any season of the year.

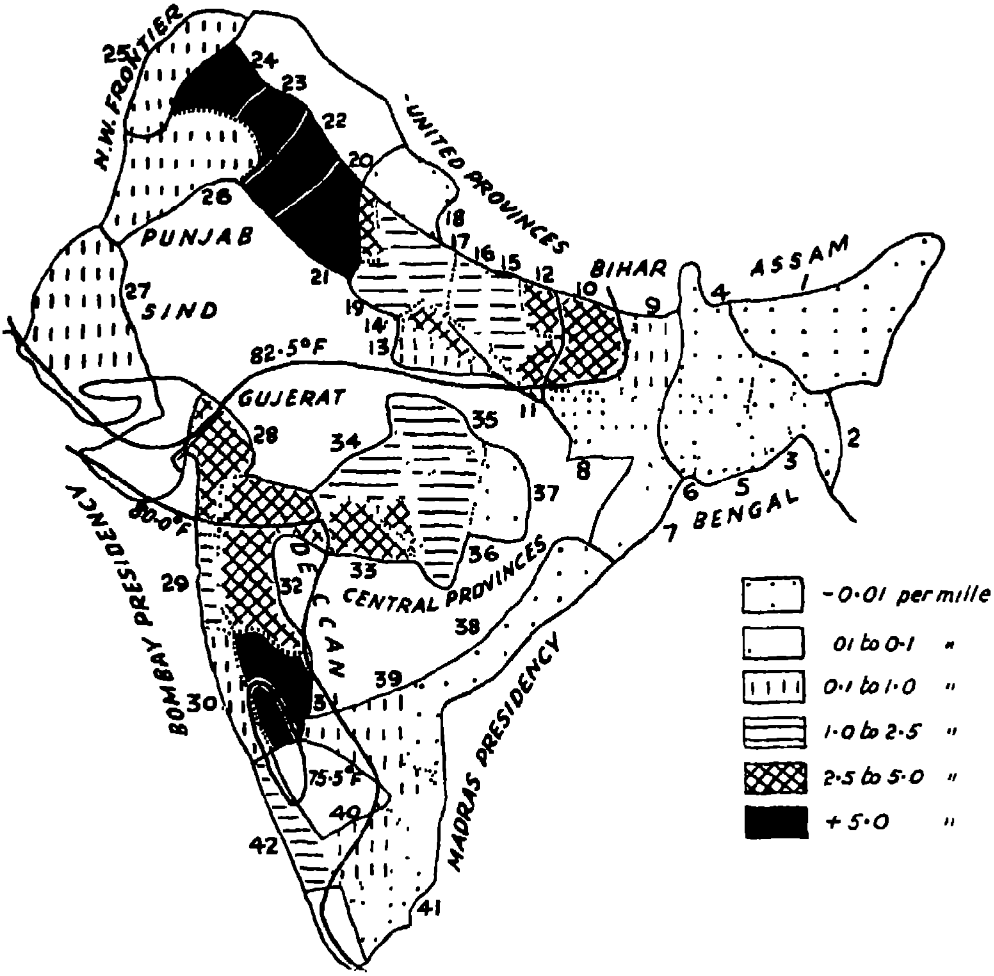
Provincial inquiries by the same body of investigators revealed the important fact that the nearly complete absence of plague from Lower Bengal, Assam and most of the Madras Presidency, other than that adjoining infected Bombay and Mysore territory, could not be explained solely on climatic grounds, but the work of L. F. Hirst (2) in Ceylon and of F. W. Cragg (3) in India has since shown that the very low plague rate in these areas is associated with the prevalence of *X. astia* in the place of *X. cheopis* as the common rat flea, the latter being by far the more efficient carrier of plague, and it should be noted that these two fleas had not been differentiated at the time of the work of the Indian Plague Investigation Committee's investigators.

Although there is thus abundant evidence regarding the influence of heat and moisture on the average monthly distribution of plague in India, I have found very little on record regarding the yearly variations in plague incidence in relation to climatic conditions, with the exception of the significant conclusion of T. H. Gloucester and F. N. White (4) in No. 83 of the Plague Investigation Committee's reports, that in the United Provinces of Agra and Oude "the association of unusual humidity during the winter months in certain districts with severe epidemics of plague is so constant a phenomenon that we feel justified in concluding that the one stands to the other as cause to effect." They regard the influence of climate as acting mainly on the life of rat fleas when separated from their host, for the longer they survive the greater the chance of their producing infection of man.

During the ten years which have elapsed since the Indian Plague Investigation Committee's work came to an end, much further material has accumulated in India in the form of the monthly and yearly plague mortalities, and a series of nearly thirty years' data are now available for comparing with the climatic records, so I have spent many months investigating the subject on similar lines to those which recently enabled me to point out how epidemics of smallpox (5) and of cholera (6) in the different provinces of India can be foreseen as a rule by watching the rainfall and absolute humidity data. The plague data are dealt with in the present paper.

The Average Annual and Monthly Incidence of Plague in Indian Provinces.

The map shows the average incidence of plague in the different divisions of each province during the twenty years from 1901 to 1920, that is, during the two decades after the disease had spread over India, and Chart 1 illustrates the yearly rates per mille for the 25 years from 1900 to 1924 in the different provinces, and it shows graphically the remarkably low incidence in Assam, Bengal, Madras and Burma as compared with the rest of India, for the data of each province given in Chart I are shown on the same scale. In the map the rates for the great cities of Bombay and Calcutta are given separately from the areas in which they are situated, as their inclusion would give a totally



Date of Map of Average Plague Deaths per Mille 1901–20.

1—Assam, 0·0013. 2, 3 and 4—Eastern Bengal, 0·00036. 5 and 6—Western Bengal, excluding Calcutta, 0·015; Calcutta, 0·140. 7—Orissa, 0·0021. 8—Chota Nagpur, 0·0357. 9—Eastern Bihar, 0·81. 10—Western Bihar, 2·89.

United Provinces Divisions—11—Benares, 5·90. 12—Gorakpur, 3·57. 13—Jhansi, 0·46. 14—Allahabad, 2·82. 15—Fyzabad, 1·70. 16—Lucknow, 2·27. 17—Rohilkund, 1·61. 18—Kamaon, 0·08. 19—Agra, 2·40. 20—Meerut, 3·27.

Punjab.—21—Amballa, 6·04. 22—Jullunder, 9·18. 23—Lahore, 10·30. 24—Rawal Pindi, 5·22. 26—Multan, 0·95. 25—North-west Frontier Province, 0·46.

Bombay Presidency.—27—Sind, 0·84. 28—Gujerat, 3·66. 29—North Konkan (excluding Bombay City), 1·11; Bombay City, 8·02. 30—South Konkan, 0·31. 31—South Deccan, 9·90. 32—North Deccan, 4·18.

Central Provinces.—33—Berar, 2·56. 34—Nerbuda, 1·41. 35—Jubbulpore, 1·56. 36—Nagpur, 2·51. 37—Chattisgarh, 0·006.

Madras Presidency.—38—North-East Coast Districts, 0·0014. 39—North Central Districts, 0·29. 40—South Central Districts, 0·83. 41—South-east Districts, 0·043 42—Malabar, 1·65.

false impression regarding the plague incidence in the North Konkan and the Presidency division of Bengal respectively, which have rates of one-seventh and one-tenth of their capital cities, with totally different housing and other conditions.

It will be seen from a study of the map that Assam, Eastern and Northern Bengal, Orissa, the North-East Coast districts of Madras and the easternly Chattisgarh division of the Central Provinces, all had rates of under 0·01 per mille, shown by widely separated dots. Western Bengal, Chota Nagpur and South-East Madras have rates of under 0·1 per mille, represented by closer dots. The next highest rates of between 0·1 and 1·0 per mille, represented by short vertical lines in the map, are met with in the widely separated areas of the most westernly Multan divisions of the Punjab, and the North-West Frontier Province, with very dry climates and great cold in the winter months, in the sparsely inhabited South Konkan West Coast districts of Bombay, the central dry elevated regions of the Madras Presidency, the southern Bundelkand division of the United Provinces, and lastly in the Eastern Bhagulpur division of Bihar bordering on North Bengal. The abrupt rise from 0·81 to 2·89 per mille on passing from Eastern to Western Bihar is very noteworthy, as there is no material difference in their climate, and the Sanitary Commissioner, E. H. Hare (7), in his report for 1921, pointed out that the districts of Bihar in which plague has never shown any signs of gaining a permanent foothold, in spite of frequent invasions from neighbouring severely affected districts, are characterised by a less dense and poorer population, living in widely separated huts built of flimsy materials with thin walls in which rats cannot congregate. Further, he states that Kunhardt found 34·4 black rats per 100 traps and an average of 8·1 rat fleas in a badly infected district of Western Bihar, but only 5·1 rats and 4·9 fleas per rat in the slightly affected Purnea district of Eastern Bihar. Similar scattered thin-walled houses and paucity of rats prevail in the nearly plague-free Lower Bengal and Assam, as described in No. 46 of the Plague Investigation Reports already referred to, and these unfavourable factors appear to be far more important than any climatic conditions in accounting for the low plague incidence in these areas. According to Hirst (2), little information is on record regarding the species of rat fleas in these areas, so the influence of that factor is not yet determined.

The remaining portions of India show the highest rates of from 1·0 to 9·9 per mille, and include the remaining portion of Bihar, the United Provinces, the Punjab, Gujerat, Deccan and the remaining four westernly divisions of the Central Provinces, all characterised by closely aggregated thick mud-walled rat-infested houses favouring a high incidence of the disease. Rates of 1·0 to 2·5 are indicated in the map by shading with horizontal lines, those between 2·5 and 5·0 per mille by crossed oblique lines, and the highest rates of over 5·0 per mille in four divisions of the Punjab and in the South Deccan by uniform dark shading. It is in these areas that the yearly variations of the disease can best be studied, and Chart 1 shows at a glance how great these yearly fluctuations are in Bihar, the United Provinces, the Central Provinces and the Bombay Presidency, the data of all of which I have studied in relation to the yearly climatic conditions. A large map, illustrating the plague mortality in each district of India from its commencement in 1896 up to 1917, will be found in the 1917 Annual Report of the Sanitary Commissioner with the Government of India. This is very similar to the smaller map accompanying this paper, only it shows in a more detailed manner the areas of high incidence.

The Seasonal Incidence of Plague in Relation to Climate.—Before considering the varying degrees of epidemic prevalence of plague from year to year, it will be well to study the average monthly incidence in the different areas in relation to the average monthly temperatures, humidity and rainfall, which are shown for eight selected areas in Chart 2, constructed on similar lines to those of my earlier studies of smallpox and cholera. In the lower halves relating to each area are shown the monthly average plague rates per 10,000 in columns, together with the monthly average saturation deficiencies. In the upper halves the average monthly rainfall is shown by vertical columns, and curves of the mean monthly temperatures are also entered.

It will be convenient to consider first the relationship of the mean temperatures to the seasonal decline and rise of the plague curve, beginning with the striking hot weather decline of the disease. In the first place it should be pointed out that the seasonal incidence of plague is very similar in the three northern areas of the Punjab, the United Provinces and Bihar, which all show a great fall and a prolonged period of low rates during the hot weather and monsoon seasons, followed by the commencement of the cold weather rise in the last month or two of the year after the complete cessation of the monsoon rains. On the contrary, in the Central Provinces and both the North and the South Deccan the usual decline of plague in the hot season is of short duration, owing to the next epidemic commencing in the monsoon period, although in the neighbouring low-lying hot Konkan Bombay coast area no increase occurs until the very end of the year, remarkable differences which require to be explained.

The Influence of High Temperatures.—On turning to the mean monthly temperature curves of the different areas, we find the main cause of both the rapid hot weather decline and of the monsoon quiescent period, where it occurs, in the rise of the mean temperature to 85° F. and over in the hot season, and its remaining at about 85° F. during the rainy season in the three northern areas, but falling well below that level during the monsoon in the Central Provinces and in both Deccan areas, namely, at just over 80° F. in the Central Provinces, just over 75° F. in the North Deccan and at between 70° and 75° F. in the South Deccan, in the last two of which the hot weather decline of plague is shortest and most incomplete, and the yearly maximum actually occurs in the late monsoon and early cold weather months. In the map already dealt with I have entered the 75·5°, 80° and the 82·5° F. mean isotherm lines for August, at the height of the monsoon, and these serve to bring out the fact that nearly the whole of the Deccan has a mean temperature below 80° and all the Central Provinces one below 82·5° F., but that the three northern areas, with continued low plague during the monsoon, have readings over 82·5° F. It is also of interest to note that in the cities of Calcutta and Bombay the mean monsoon temperature is between 80° and 85° F., accompanied by low saturation deficiencies favourable to plague, and here the disease continues at about the same level as at the end of the hot weather decline, which is less complete than in the hotter northern areas, although in all the areas the saturation deficiency is at its highest level favouring plague during the moonsoon period, so that the mean temperature is clearly a most important factor influencing the monsoon level of the disease.

Humidity.- Here we may consider the absolute humidity or mean vapour tension, which proved the key to the climatic influence on smallpox and cholera, or the relative humidity and the saturation deficiency, which run a parallel course, as they both depend on the degree of saturation of the atmosphere with moisture at the particular temperature. On plotting out the average monthly curves for each area, and studying the yearly variations of the absolute humidity and the saturation deficiency, I found that the latter showed the closer relation to the plague curves, so in Chart 2 I have entered the saturation deficiency curves of each of the eight areas dealt with, and have also worked out the relative humidity data, which served to bring out the striking resemblances between the two. Unfortunately the monthly relative humidity data for various stations in India have for long been omitted from the Indian sanitary reports, so for my studies of the yearly variations of plague in India I have worked out the mean monthly saturation deficiencies for about thirty years in each area, which were obtained by deducting the monthly absolute humidity data, kindly supplied me by the Meteorological Department of India, from the saturation point at the mean monthly temperatures of a central place in each area, as the monthly temperature and rainfall data are still recorded in the Government of India Annual Sanitary Reports.

Saturation Deficiencies and the Seasonal Incidence of Plague.—It will be convenient to consider first the average monthly saturation deficiencies plotted out in Chart 2 in relation to the average seasonal incidence of plague. It will be seen at once that all the areas, exclusive of the very humid cities of Bombay and Calcutta, show a great increase of the saturation deficiency, indicating low degrees of humidity, in the hot weather season of March or April to June, accompanied or immediately followed by the great annual decline of plague. Moreover, this decline of plague will be seen to be most complete in the Punjab, the United Provinces, the Central Provinces, the North Deccan and Bihar, that is, just those areas where the hot weather saturation deficiency reaches the highest degrees of from nearly 1·200 in the Central Provinces down to 0·700 in Bihar. On the contrary, the decline is least complete in Bombay City with a saturation deficiency never much below 0·300, showing the highest humidity at this season of all the areas. High degrees of saturation deficiency, indicating low relative humidity, therefore play an important part in aiding high temperatures to bring about the hot season decline of plague, as Brooks pointed out.

The Seasonal Increase of Plague and the Climatic Factors.—The three northernly areas of the Punjab, the United Provinces and Bihar (the data of the last having been worked out for Bihar proper, exclusive of Chota Nagpur and Orissa) all show a cold weather increase of plague, coinciding with increased saturation deficiency and increased relative humidity, due to the smaller quantity of moisture required to saturate the air at the comparatively low temperatures now prevailing, and as both the higher relative humidity and the lower temperatures are favourable to plague, the average monthly data throw little light on which of these two factors most influences the cold weather incidence of plague. Both factors, therefore, require to be studied in relation to the great variations in the prevalence of plague from year to year in the different geographical areas illustrated in Chart 2, which have been selected as having high plague rates and varying climatic conditions.

Yearly Provincial Plague Incidence in Relation to Climate.

For the purposes of the following inquiry I tabulated from thirty years' annual sanitary reports of the Government of India the monthly plague mortality figures, the monthly mean temperature and the rainfall of the areas dealt with, and also the monthly absolute humidity data for working out the saturation deficiencies. In the case of Bihar and of the South and North Deccan the monthly plague data had to be calculated from the district figures, and for all areas the monthly rates per mille were estimated year by year to enable the high and low rates to be noted, the data numbering over 20,000. Next the temperature, rainfall, absolute humidity and saturation deficiency data were worked out for each of the four seasons for every year, and the influence of each factor on the monthly and yearly variations in the plague mortality of each area studied in relation to those data. By this means it was ascertained that the monthly mean temperatures during the hot weather and the monsoon periods had a definite effect on the monthly and yearly plague mortality from year to year, and that the saturation deficiencies in those two periods, and also during the two cold weather periods of from October to December and of January to February or March also had a definite effect, making six factors in all requiring detailed study, but the uniformly favourable cold weather temperatures appeared to have little or no effect, so could be omitted from the following charts and tables without loss, and with some degree of simplification of a complicated inquiry. Further, the annual rainfall was divided into the same four seasons for the purpose of tracing its effect on the humidity and temperature variations. The two highest plague areas of the Punjab and the South Deccan, illustrating the greatest variation in the climatic conditions, have been selected for charting all the above-mentioned factors, and owing to the time required to follow all these curves year by year the two temperature and four humidity data have been tabulated for years of increasing and decreasing plague for those two areas, as well as for the United Provinces, Bihar and the Central Provinces, and the yearly plague incidence of each province has already been illustrated by Chart 1, in which the figures are included in those of Bombay.

The chart of the Punjab will be first considered in detail as representative of the three northern areas with a winter rise of plague, followed by an analysis of the tables of the data of the United Provinces and Bihar. Similarly, the chart of the South Deccan will be dealt with fully, as representative of the three more southern areas with a monsoon increase of the disease, and a table of the Central Provinces will also be considered.

The Punjab.

The climatic variations of the Punjab are illustrated by Chart 3, which contains curves and columns illustrating the following data. In the right-hand part of the chart (3, *b*) the total and seasonal variations in the rainfall are shown below. The full height of the columns represent the total annual rainfall, and that of the different seasons is shown by various shading thus : the dark shading at the bottom of the column is that of the cold weather of January to March, the oblique lines that of the hot weather from April to June, the vertical lines that of the monsoon period from July to September, the monsoon being of shorter duration in the Punjab than elsewhere in India, and, lastly, the upper dark shading is that of the cold season from October to December. The columns above the rainfall data in 3 (*b*) and at the bottom of 3 (*a*) show the yearly total plague mortality, and it is repeated to enable the temperature curves above in 3 (*b*), and the saturation deficiencies in 3 (*a*), to be followed year by year in relation to the plague incidence.

Yearly Variations in Rainfall in Relation to Temperature and Saturation Deficiency.—An examination of the data in Chart 3 shows a general relationship between high rainfall and low temperature and low saturation deficiency, indicating high relative and absolute humidity, as might be expected. For example, high monsoon rainfall occurred in 1900, 1908, 1909, 1913, 1916 and 1917, and all showed exceptionally high humidity represented by low saturation deficiencies in the monsoon period from July to September ; and the low monsoon rains of 1904, 1911, 1915, 1918 and 1920 were all accompanied by high saturation deficiencies and low humidity. In the years of fairly average rainfall, however, such a close relationship is not found to the mean monthly humidity, for heavy falls on only a few days in the month will have much less effect than a more general distribution of the rain with its accompanying cloud. The mean monthly temperature is also higher with little rain and low with well-distributed heavy monsoon falls.

In the hot season from April to June very similar relationships hold good, only here the temperature is a most important factor influencing the saturation deficiency owing to the small amount of rain at this period, except occasionally late in June in years of unusually early extension of the monsoon to the Punjab. Thus, the greatest hot weather rainfall in 1909, 1913 and 1917 was in each instance accompanied by exceptionally low temperatures and saturation deficiencies favourable to plague, and nearly all the years of very low hot weather rains were associated with unfavourable high temperatures and saturation deficiencies. 1920 was an exception with low saturation deficiency in spite of low rainfall, and this was due to a most unusual defect of 6·7° F. in the mean temperature of May and smaller ones in April and June, showing the great importance of temperature in the hot season.

In the cold weather periods of low rainfall at the beginning and end of the year there is also a close general relationship between high rainfall and increased humidity shown as low saturation deficiency, and vice versa, and the exceptions are again explained by unusual temperature variations, and they only occurred very occasionally in the October–December period, as in 1909, 1916 and 1917 due to an excess of 4·4° F. in the former year in November, and a defect of 4·5° F. in the same month of 1917 and of 3·4° F. in 1916. In the remaining cold weather season of January to March there was a uniformly close relationship between high rainfall and high humidity in every year except 1908, 1912 and 1913, in each of which the comparatively high saturation deficiency was accounted for by high temperatures in January or February or in both months. Thus in all three seasons of low rainfall the temperature variations have to be taken into account as well as the rainfall in explaining the saturation deficiencies, indicating once more the importance of the study of the latter data in relationship to plague incidence if accurate forecasts are to be made.

The Yearly Variations in Temperature in the Hot Seasons.—The yearly temperature variations from the average for the monsoon months from July to September, and for the early hot weather months of March and April are shown in the upper part of Chart 3 (*b*), the minus readings above and the plus ones below the horizontal line representing the average readings, the former being favourable and the latter unfavourable to plague incidence. The monsoon temperature data are entered in continued lines, and the variations influence the plague incidence in the following year, while the March–April readings, represented by broken lines, affect the disease in the same year, as high temperatures bring about a more rapid hot weather decline of plague. The data are obtained by dividing the total excess or defect of the mean temperature of each period by the number of months included in them, and the mean monthly readings varied between 77° and 90° F. in the four monsoon, and between 69° and 80° in the two spring months. It will be noted that the yearly variations are very considerable, and they greatly exceed the similar data of the South Deccan shown in Chart 4 (*b*), especially in the monsoon period, owing to scanty rainfall more often occurring in the Punjab in this season. The temperature in May and June averages from 89° to 94° F., which is too high to allow of any material plague incidence, and those of the cold weather months are too low to be unfavourable to the disease, so their yearly variations have not been charted, as they show less close relationship to the prevalence of the disease.

Yearly Variations in the Saturation Deficiencies at Different Seasons.—In Chart 3 (*a*) the transverse lines show the average saturation deficiencies in the four seasons dealt with, and the years in which the curve rises above the average indicate low saturation deficiencies or high humidities, and vice versa. Those of the lower three curves shown by continued lines of the hot weather, monsoon and early cold weather months from April to December influence the plague of the following year, and the upper curve in dotted lines of the first three months of the year influence the disease in the same year. The figures of hot weather months from April to June average 0·772, and those of the monsoon period from July to September average 0·511, high degrees occurring when the temperature is also high. On the other hand, the cold weather averages are 0·321 from October to December, and 0·241 from January to March, or readings favourable to plague incidence and occurring during the increased seasonal prevalence of the disease. We thus have four saturation deficiencies and two temperature curves to consider, and owing to their number I have worked out Table I to show the favourable and unfavourable factors in the years of increasing plague in the upper half, and those of decreasing disease in the lower half, so as to allow the importance of each factor to be seen at a glance without the necessity of tracing each curve for every year to ascertain their influence. Major variations from the normal are shown by — — when the temperature or saturation deficiency is low, and thus favourable to high plague incidence, and by + + when high and unfavourable to the prevalence of the disease, and by a single sign when the difference from the normal is not so great. A blank indicates that there was no material variation from the normal.

Table of the Climatic Factors influencing Plague Incidence in the Punjab.—It will be seen from Table I that in the seven years of increasing plague, shown in the upper part, all the signs, except one of minor degree, were minuses, indicating low temperatures and saturation deficiencies favourable to plague. Similarly, in the lower part of the table, showing six years of great decrease of the disease, the great majority of the signs are plus, representing high temperatures and saturation deficiencies unfavourable to the disease, and only one of the contrary signs is of the major degree. Further, of the seven contrary signs in the whole table three fall under the March–April temperature, which only influences the termination of the annual rise of plague, and two others occur in April-June and one in the January–March saturation deficiencies. This leaves only one minor contrary sign in the three most important factors for forecasting plague epidemics, namely, the temperature and saturation deficiency in the monsoon months, and the latter factor in October to December, for the data of the first two are available before plague begins to rise in this province, and the last before any material rise has taken place. The table, therefore, affords very strong evidence regarding the influence of all six factors on plague incidence, and this is greatly enhanced when the monthly variations of the disease are examined in detail, for I find that in every case when the climatic variations were of the major degree, their influence could be traced in just those months the particular factor might be expected to influence most, examples of which will be given later.

Table I.—Climatic Factors influencing Plague Incidence in the Punjab.

Year.	Data of previous year				Data of same year.	
	Temperature.	Saturation deficiencies.			Temperature.	
	July–October.	April–June.	July–September	October–December.	January–March.	March–April.
		Years of Increasing Plague.				
1904			—		—	—
1907			—		— —	— —
1910	—	—	—			— —
1914	—	—				—
1915	—	—	—	—		+
1918	—	— —	— —	—	—	— —
1924	—		—			
		Years of Decreasing Plague.				
1906	+ +	+	+	—	—	—
1908	+	— —	+ +	+ +	+ +	
1912	+ +		+ +	+	+	—
1916	+ +	+ +	+ +		+ +	+ +
1919	+		+ +	+		
1921	+	—	+ +	+ +	+ +	+ +

Thus the influence of low temperatures and saturation deficiencies in April to June in favouring plague is seen in comparatively little fall in May or even in June, in consequence of which the decline is not as great as usual in the monsoon period, and the disease is liable to be carried over to the next cold season. Similarly, favourable conditions in the monsoon period, due to heavy rains, are shown in an early rise in the late monsoon and early cold weather months followed by a great rise in first quarter of the succeeding year.

Again, in the six years of greatly decreased plague high temperatures and saturation deficiencies were present in the monsoon period, July to September, in every one, as shown in lower part of Table I, and in 1916 and 1921 both factors were also unfavourable in the first four months of those years, with the result that coming on the top of the unfavourable monsoon factors of the previous year, the plague rates fell to the lowest rate in the whole of the twenty-four years since the disease became well established in the province. In view of these striking facts it is unnecessary to go through the wearisome task of analysing each of the six curves in detail, as it will suffice for my purpose to explain the most striking yearly variations in plague incidence shown in Chart 3.

Analysis of the Principal Punjab Plague Variations.—Plague first became prevalent in part of the Punjab in 1901 with a mortality of 0·84 per mille, which rose to 8·78 per mille in 1902 with further extension of the affected areas, and it became generally prevalent throughout the province in 1903 with a death-rate of 9·60 per mille, only a slight increase on the previous year owing to the climatic conditions not being especially favourable. In 1904 the mortality jumped to 19·82 per mille with favouring low saturation deficiencies in the previous monsoon period and in January–March, 1904, as well as low temperature in March and April, which resulted in the second highest May and June rates of the series of years, fully accounting for the great rise. 1905 also showed the high rate of 16·65 per mille, with favouring humidity and temperature in the early months of the year. In 1906 a remarkable drop to only 4·56 per mille took place, followed by the record rise, showing that the initial influence had not exhausted itself, so the temporary 1906 decline is of especial interest. The chief factors in bringing this about were high saturation deficiency, accompanied by the record high temperature, in the monsoon period of 1905, coming on the top of high temperatures in April to June, which had brought about a decrease of plague in June, 1905, to one-tenth of the May rate, with the result that the disease remained at a low rate throughout the second half of 1905, and in the usual maximum season early in 1906 it remained far below the level of the previous four years. The 1906 decline is thus fully accounted for by these unusually unfavourable climatic features, all of which occurred several months before the main annual rise of the disease, so that with a knowledge of the factors I have now worked out this extraordinary decline might have been foreseen.

In 1907 plague reached its maximum in the Punjab with 30·27 per mille, or 608,685 deaths from this disease alone in one province of India, and this great increase was favoured by the following climatic conditions. In the first place, low temperature in March to April prevented the usual decline of plague in May, and low saturation deficiency in July to September also favoured the carrying over of plague through the monsoon period, with resulting unusually early increase in September, when it is low in normal years, and a steady increase during the last four months of the year, to reach a comparatively high point by December. On the top of these favouring conditions came very low saturation deficiency in January to March, 1907, accompanied by a rapid rise of plague in the usual season, and this was accentuated still further by the record low temperature of the whole series of years in March and April, with the result that the disease reached unprecedented heights in April and continued at the same extreme level in May, in place of the usual decrease of that month, and both June and July also showed record rates, fully accounting for the phenomenon dealt with.

Fortunately the climatic factors now became equally unfavourable, resulting in the record decline of plague in the following year, 1908, with a rate of only 1·53 per mille, and this was due to unfavourable high saturation deficiency continuously through the three seasonal periods of from July, 1907, to March, 1908, with especially high rates in the first and third periods charted, while the temperature was also unfavourably high in the monsoon period of 1907; fully accounting for the exceptionally low plague rate in 1908 in spite of a high incidence up to September, 1907.

The rate remained at the low figure of 1·77 per mille in 1909 with nearly normal climatic features, but in 1910 it rose once more to 6·74 per mille with favouring low temperatures at both periods, and also two periods of low saturation deficiencies, and in the absence of any unfavourable climatic factors the rate continued at the relatively high figure for the period subsequent to the great initial epidemic of 8·89 per mille. In 1912 plague declined once more to only 1·54 per mille with slightly favouring low temperature in the hot weather of 1912, but with four of the remaining five climatic factors unfavourable to the disease, and a second low year ensued in 1913 with two unfavourable and one favourable factor. In 1914 a slight increase to 3·31 per mille accompanied favouring low temperatures at both periods and nearly average saturation deficiencies, except for one favouring rate, but in 1915 the incidence rose greatly to 11·48 per mille as the result of favourable low temperature from June to October, and low saturation deficiencies continuously during the three periods from April to December, 1914, with the result that the moderate rise of 1914 was carried on to 1915 in an accentuated degree. Fortunately, this epidemic rise was checked by a slight excess of temperature in March and April, 1915, followed by all the six factors from the hot weather of 1915 to April, 1916, being highly unfavourable, with the sole exception of a normal saturation deficiency in October to December, with the result that 1916 showed a fall to the record low rate of 0·17 per mille. In 1917 there was a slight rise to 9 45 with favouring saturation deficiency in the monsoon period and favourable low temperatures, but 1918 showed a considerable increase to 4·94 per mille, with both temperature and three saturation deficiency readings from April to December inclusive low and favourable to the disease. In 1919 the plague rate fell again to 0·57, as the result of high temperature and saturation deficiencies in the important monsoon period, and continued low humidity in October to December, and no factors favourable to the disease, and it reached the record low figure of 0·13 per mille in 1921, with all the factors unfavourable to plague except slightly low saturation deficiency in the hot season, and four of the factors highly unfavourable.

Lastly, a moderate rise took place to 2·24 per mille in 1923 without the occurrence of any strikingly favourable climatic conditions, and the rate increased greatly once more to 12·24 per mille in 1924, accompanied by favouring low temperature in July to October, 1923, and low saturation deficiency in the same monsoon period, and no unfavourable climatic factors, with the result that the rise in 1923 was carried over the monsoon period, during the latter part of which it was at a higher rate than in any year since the initial great epidemic ones, and became epidemic once more in the early months of 1924, no less than seventeen years after the first epidemic reached its height in 1907, indicating that further epidemics are not unlikely when the climatic conditions favour a recrudescence of the disease.

It will also be observed that the 1924 outbreak caused the highest plague mortality since 1907, yet the climatic factors were only moderately favourable, so it seems probable that it was also favoured by the prolonged quiescent period in 1919 to 1922 inclusive, possibly by allowing of the accumulation of a large percentage of susceptible rats, for it is well known that after an epidemic many of the rats are for a time insusceptible to the disease.

Summary.--The foregoing detailed analysis shows that the yearly incidence of plague in the Punjab can be explained very well by the climatic factors I have indicated being favourable or unfavourable as the case may be. Further, their influence can be traced on the monthly distribution of the disease, the rises or falls being in just those periods and in the direction which the particular abnormal factor might be expected to produce, so the analysis affords striking evidence of the importance of the yearly variations in the climatic conditions in influencing the monthly and yearly incidence of plague in the Punjab. Further, the fact that no less than four of the six factors become apparent during the eight months previous to the seasonal rise of the disease in December or January should enable the epidemic increases and the decreases of the disease to be foreseen in the future with a fair probability of accuracy, and this makes the study a matter of some practical importance. The two remaining factors operating from January to April affect mainly the height of the epidemic rise in the early months of the year, so they are of value in determining the duration of the high annual incidence. As the Punjab shows the highest plague incidence in the northern areas of India, and also has the greatest yearly climatic and plague variations, it is the most important area of this region for such a study, and the United Provinces and Bihar, with similar seasonal distribution of the disease to the Punjab, can be dealt with more briefly to ascertain if the same influences affect the yearly variations in them also in a similar manner.

United Provinces of Agra and Oude.

The United Provinces stretch along the Ganges valley from the Punjab in the west to Bihar in the east, and Chart 2 shows that the average monthly prevalence is similar in all three, with the exception that the maximum season lasts longest in the Punjab and ends earliest in Bihar, in accordance with the later rise of the unfavourable hot weather temperature from the south-east to the north-west. Moreover, both the yearly variations in the plague incidence, shown in Chart 1, and the climatic factors of temperature and humidity vary most in the Punjab and least in Bihar. All three areas derive their rainfall largely from the monsoon current sweeping up the Ganges valley from the Bay of Bengal from June to October, but the western divisions of the United Provinces and the Punjab also receive some from the Bombay branch of the monsoon current, so it is not surprising that the climatic factors already shown to influence the yearly plague incidence in the Punjab are also operative in the United Provinces and Bihar. I have therefore worked out the same climatic factors for the two more easterly areas, and have made out tables on similar lines to Table I of the Punjab, which suffice to demonstrate their influence in a simple and easily demonstrated manner.

The recorded plague incidence in the districts of the United Provinces show that the disease took several years to become disseminated throughout this extensive area, and this is illustrated by the step-like rises of the total annual incidence from 1902 to 1904 in Chart 1, but by the end of 1904 this process had been completed, so we can study the influence of climate on the disease from 1905 onwards. The first part of Table II shows the variations in the six climatic factors already described on plague prevalence in the years of considerable increase of the disease on the previous year, and it will be seen at

Table II.—Climatic Factors influencing Plague Incidence in the United Provinces.

Year.	Data of previous year.				Data of same year.	
	Temperature.	Saturation deficiencies.			Temperature.	
	July–October.	April–June.	July–September.	October–December.	January–March.	March–April.
		Years of Increasing Plague.				
1905	− −			−		− −
1907		+ +			− −	− −
1910	−	− −	−	−	+	
1911		− −		−	− −	− −
1917	−	−	−	−		− −
1918		−		−	+	
1923		+ +				+
		Years of Decreasing Plague.				
1906	+	+		+ +	−	−
1908	+ +		+ +	+ +	+	+
1912	+ +	−	− +	− −		−
1919			+ +	−	+ +	−
		Years of Continuing Low Plague.				
1909		+		+	+ +	−
1916	+			+	+ +	+
1920		+ +			−	
1921		+		+ +	+ +	+ +
1922		+ +	−	+	+	+

once how closely the data correspond to those in Table I of the Punjab. This is due to the fact that four of the seven years showed a great increase in both provinces simultaneously, namely, in 1907, 1910, 1911 and 1918, and two of the remaining years also showed high rates in both provinces. The only exceptions are 1917 with a moderate increase of plague in the United Provinces alone, and 1915 with a rise of the disease in the Punjab only, and these are explained by an excess of the monsoon rain, with the usual accompaniment of low temperatures and saturation deficiencies favourable to plague, in the Punjab alone in 1914–15, but a considerable defect of the monsoon in the United Provinces; while in 1916 there was excess of rain in July only in the Punjab, but in June and August to November inclusive in the United Provinces, with the usual effect of increasing plague in the following year.

It will also be noted that in Table II low saturation deficiencies of April to June are not so constant in the years prior to plague increases as in the Punjab. This is due to the temperature not rising so high in the latter province, and Table III shows that this factor has still less influence in Bihar with a still milder hot season. With that slight exception the causes of increased plague are the same in all three areas.

On turning to the years of considerable decrease of plague in the United Provinces in the middle part of Table II, it will be observed that all four years also showed a similar great decline in the neighbouring Punjab, and this was due to the same causes of unfavourable high temperatures and saturation deficiencies, those of April to June again showing less influence in the more easterly United Provinces with less excessive heat at this period, so it is unnecessary to follow the data in detail. Once more, the years of continued low plague in the last part of Table II show that in these years the unfavourable climatic factors predominated greatly over the favourable ones, but they less frequently showed the major degrees represented by + + except in the case of the saturation deficiencies of January to March, which comes too late to do more than lessen the height of the late cold weather rise of plague.

Summary.—The climatic factors influencing the yearly variations of plague are essentially the same as in the Punjab, and due to the monsoon rains affecting both provinces, and they are equally valuable in allowing very probable forecasts of the disease to be made several months before the annual rise of the disease.

Bihar.

In the Bihar area the extent of the yearly plague variations, as shown in Chart 1, is again less than in the neighbouring more westerly United Provinces on account of the seasonal rainfall, humidity and temperature showing greater uniformity. Table III shows the temperature and saturation deficiencies in years of increasing, decreasing and of continued low incidence of plague, as in the last table, except that the hot weather saturation deficiency is calculated for April and May only, because the earlier onset of the monsoon lowers that of June below the level unfavourable to plague, and the average reading for April and May is only 0·651 in Bihar against 0·815 and 0·792 in the three hot weather months of the United Provinces and the Punjab respectively. In consequence this factor has comparatively little influence in Bihar, as shown by column 2 in Table III, and should be omitted in forecasting epidemics there, but it has been entered to allow of comparison with the other provinces.

With the omission of the April and May saturation deficiencies, in the eight

Table III.—Climatic Factors Influencing Plague incidence in Bihar.

Year.	Data of previous year.				Data of same year.	
	Temperature.	Saturation deficiencies.			Temperature.	
	June–October.	April–May.	July-September.	October–December.	January–March	March–April.
		Years of Increasing Plague.				
1901	−	+	− −	−	− −	−
1903				−		
1905	−	−		−		− −
1910	−	−				−
1911	− −		−	−		− −
1914				−	−	− −
1917	−	+		−		−
1923	−					
		Years of Decreasing Plague				
1902	+ +	+ −	−	+	+ +	+
1906		−			− −	−
1908	+	−	+	+ +		
1915	−	− −		+	− −	−
1919		− −	+ +	− +		
1924		+			+	+ +
		Years of Continued Low Plague.				
1909	+	+	+ +	+ +	+ +	−
1916	+	−			+	
1920	−	+				−
1921	+	+ +	−	+ +	− +	+
1922		+			+	+

years of increasing plague only one unfavourable climatic factor of minor degree remains, and the years of decreasing plague show a considerable preponderance of unfavourable climatic factors (except in the April–May saturation deficiency as just explained), especially among the remaining three operating in the previous year, which are of the greatest consequence in forecasting plague, for those of January to April only affect the height of the seasonal rise at that time, and not the origin of the epidemics. Further, the years of continued low plague at the bottom of the table also show a considerable majority of factors unfavourable to the disease, and they were particularly well marked in 1909 with the lowest Bihar plague mortality in the twenty-five years dealt with. On examining tables of the monthly incidence I also found in both Bihar and the United Provinces a close correspondence between the particular favourable or unfavourable climatic variation and the rises and falls of the monthly incidence as compared with the averages for each month, leaving no doubt regarding their effects in these areas as in the Punjab.

Summary.—The climatic influences on plague incidence in Bihar are similar to those in the United Provinces and the Punjab, except that owing to the milder hot weather and lower saturation deficiencies in April and May this factor exerts comparatively little effect on the curves, so very probably forecasts are also possible in Bihar.

Central Provinces.

The Central Provinces lie between the United Provinces and Bihar to the north-east and the North Deccan area of the Bombay Presidency to the south-west. The plague prevalence is of special interest in this area owing to its having the seasonal incidence of the Deccan but the hot weather temperatures of Northern India, as shown in Chart 2, due to the high hot weather temperature and saturation deficiency bringing down the disease to a very low level, but the monsoon temperature is not high enough to prevent a recrudescence once more in August to October, much earlier than in the northern areas with higher monsoon temperatures, and the disease continues to be prevalent throughout the cold weather up to March. A single epidemic therefore extends over the latter part of one year and the early part of the second, necessitating the study of the plague data from June of one year to May of the next, and I have worked out the necessary data in the place of the annual rates. The comparatively low plague incidence in the Central Provinces is mainly due to only the western divisions on the Deccan side having high plague rates, as shown in the map. It will also be observed from Chart 1 that the epidemic rises in the Central Provinces occur in the same years as a rule as in the Bombay Presidency, and the latter curve is essentially the same as that of the Deccan, which constitutes the largest, the most populated and the most plague-stricken portion of Bombay as a whole, as shown in the map.

Analysis of the Yearly Plague Incidence in Relation to the Climatic Factors.—The six climatic factors influencing plague are given in Table III for all the years from 1903–04, when the disease had become generally prevalent, up to 1920–21, after which there were no material variations in its prevalence, so the following analysis will enable the influence of climate on its incidence to be traced. Owing to the hot weather decline of the disease occurring earlier here than in Northern India, the variations in the temperature in April to June

Table IV.—Climatic Factors Influencing Plague Incidence in the Central Provinces.

Year.	Data of first year.					Data of second year.
	Temperature.		Saturation deficiencies.			
	April–June.	July–October	March–June.	July–September	October–December.	January–February.
			Years of Increasing Plague			
6.03–5.04		+		− −		
6.06–5.07	+		+	− −	−	− −
6.09–5.10	− −		− −	−		−
6.14–5.15	− −		− −	−	−	−
6.16–5.17	−				−	− −
6.19–5.20	− −	−	− −		− −	−
			Years of Continued High Plague			
6.10–5.11	+			−	− −	
6.11–5.12			+	+	−	− −
6.15–5.16	+			+	− −	
6.17–5.18	−		− −	− −	−	
			Years of Decreasing Plague.			
6.04–5.05				+	−	
6.07–5.08	− −	+	− −	+	+ +	
6.12–5.13	+ +		+ +	+		
6.18–5.19	− −	+	− −	+ +	+ +	
6.20–5.21	−	+	−	+ +	+ +	+ +
			Years of Continued Low Plague.			
6.05–5.06	+				+	+
6.08–5.09	+ +	−	+ +		+	+
6.13–5.14		+		+	+ +	+ +

mainly affect the carry-over of plague to the following monsoon rise of the disease, so the data are placed in the first column, but otherwise the table is constructed on similar lines to the previous ones. The years in which plague continued low or high are entered, in addition to those in which it rose and fell, so as to allow a continuous series of seasonal curves of plague to be studied.

It will be seen at a glance that in both the years of increasing disease and in those of continued high prevalence, the favouring climatic factors of low temperature and low saturation deficiency greatly preponderate, and it is noteworthy that none of the few opposite signs are of major degree

Similarly, in the years of decreasing or continued low plague in the lower half of the table, unfavourable high temperatures and saturation deficiencies predominate to a large extent, except in the first and third columns relating to the climatic conditions in the hot season from March to June, which is also the season when two of the three adverse signs in the upper half of the table occur, so the temperature and humidity of the hot season is less closely related to the yearly variations of plague than the data from July to February, much as we found to be the case in Bihar. The significance of this will appear in the following analysis of the yearly variations, in which the influence of the different factors on the monthly incidence will be mentioned, as in the case of the Punjab.

It was not until 1902-03 that plague became widespread in the Central Provinces, with a rapid increase from December to April, coinciding with decreased saturation deficiency from October to February. In 1903-04 there was a further rise from 3·02 in the previous season to 4·84 per mille, which began in August with low saturation deficiency from July to September and also in October to December. 1904-05 showed a decrease to 1·25 per mille, with only moderately high saturation deficiency in the monsoon period, and low readings in October to December, so this appears to be swinging back after the initial severe outbreak, as a similar decline took place at the same time in the neighbouring North Deccan. In 1905-06 the disease remained low, with three adverse climatic factors and no favouring ones.

1906-07 showed a rise of plague mortality once more to 3·26, for although the incidence was very low in June and July in relationship to moderately unfavourable temperature and humidity, but with favouring factors during the last four periods from July to February, the disease increased considerably in the last five months of 1906, and showed a great rise in the first four months of 1907 with high humidity.

In 1907-08 plague declined to the lowest rate in the first ten years of its prevalence, namely, 0·79 per mille, in spite of the disease having been unusually prevalent for each month up to September, owing to very favourable low temperature and saturation deficiency in the hot season of March to June, 1907. Fortunately, both the temperature and humidity were unfavourable during the monsoon period, and this was followed by exceptionally unfavourable high saturation deficiency from October to December, with the result that the disease declined in a most unusual manner for this season and remained low in the early months of 1908, a remarkable example of unusual monthly incidence being at once explained by the climatic factors under consideration. Moreover, plague continued at the low point of 0·80 per mille during 1908-09, with the lowest hot weather rate of the first ten years of plague coinciding with very unfavourable temperature and humidity, and also low rates early in 1909 with unfavourable winter humidity.

In 1909–10 plague began to increase as early as August, and continued high up to February, with a rise of the mortality to 2·69 per mille, due to both the temperature and three of the four humidity periods being favourable to the disease. The rates remained high during the next two years, with 2·10 and 2·05 deaths per mille, with three favouring and one unfavourable factor in 1910–11, while in 1911–12 there was low prevalence in the hot weather and early monsoon period, due to moderately unfavourable saturation deficiencies from March to September, but high degrees of humidity in the two successive periods from October to February again resulted in plague rates above the monthly averages from November to April, in accordance to expectations.

1912–13 showed a remarkable decline to only 0·07, and in 1913–14 plague was practically absent, with under 0·01 per mille, and it is very noteworthy that in neither of these two years was a single one of the climatic factors favourable to plague, while three in the first and four in the second year were unfavourable, two in each year being of the major degree.

In 1914–15 the plague mortality rose again to 0·89 per mille, with five favourable and no unfavourable climatic factors, and in 1915–16 there was a further increase to 1·35 per mille, with very favourable humidity conditions in the October to December period, when the monthly death rates first rose above the average, and this rise followed low rates in the earlier months with moderately unfavourable climatic conditions In 1916–17 a further striking rise to 3·24 per mille occurred, with four favouring and no unfavourable climatic factors, and it is again noteworthy that the highest mortality occurred coincidently with the particularly low saturation deficiencies during the two successive periods from October to February inclusive.

1917–18 was distinguished by showing the lowest temperature and saturation deficiencies in the hot season from March to June of the whole series of years, in relation to which plague continued at the highest level for those months of the twenty years analysed, and it continued to increase to the end of the year coincidently with favouring climatic conditions from July to December, and the rate for the year continued at the high level of 2·31 per mille.

In 1918–19 the rate declined to 0·18, for, although the hot season was favourable to the disease, yet from July right on to December the humidity was exceptionally unfavourable to the disease, and a slow rise from July to October as usual was converted into a fall in November, when the disease usually increases, and favouring humidity in January and February caused only a slight rise, the whole year being a low one.

In 1919–20, with five out of the six climatic factors favourable to plague, a sharp rise to 1·34 ensued, only to decline again to 0·30 in 1920–21, with moderately favourable climate in the hot season resulting in a rise to a relatively high rate in August; but major degrees of saturation deficiency throughout the three periods from July, 1920, to February, 1921, brought the mortality down to the lowest of the whole series of years with the exception of 1913–14. During the last three years from 1921–22 to 1923–24 plague continued at a fairly uniform low rate, with the climatic variations for the most part of a moderate degree, and mostly unfavourable to the disease, accounting for the absence of any epidemic rises.

Summary.—The foregoing analysis is in complete accordance with those of the three northern areas in showing both a close relationship between favouring climatic conditions and increased plague and vice versa, and also in demonstrating that the variations of the mortality rates at any particular season are in accordance with the major variations in the corresponding climatic conditions. Moreover, as the two hot weather factors, and to some extent the monsoon ones, are apparent before the main seasonal rise of plague takes place from September to March, it should be possible to foresee the epidemic rises to a considerable extent in this province, although not so fully as in the more northernly areas previously dealt with, in which the yearly rise does not commence until December or later.

The Deccan

There remains among the badly infected plague areas of India the Deccan Plateau between the Western Ghats and the Central Provinces and Hyderabad State to the east. It is characterised by a very mild hot weather and low monsoon temperature, resulting in a very short and incomplete hot weather remission of plague, as shown in Chart 2, and these features are especially marked in the South Deccan districts of Belgaum, Bijapur and Dharwar, where the maximum yearly incidence is in September to November; but the rates continue high up to February, explaining the fact that this area has the highest average plague incidence in India, as shown in the map. I have therefore shown the yearly variations of plague and the climatic factors in Chart 4, constructed on similar lines to Chart 3 of the Punjab.

Rainfall and Temperature and Humidity.—The rain in the cold weather months of January and February is usually absent, and it only exceeded 0·12 of an inch in the five years with dark shading at the bottom of the columns in the lower part of Chart 4 (*b*), in all of which the saturation deficiency was low, indicating high humidity. In the hot weather months of March to May the fall varied between 1·02 and 10·42 inches, and is shown by oblique lines, and the three years of highest rainfall showed high humidity, and the three with the lowest rainfall all recorded low humidity. The temperature is an important factor in this season, years of high temperature as a rule showing low humidity and vice versa. A very large proportion of the annual rain falls in the monsoon months from June to September, and is shown by vertical lines, and heavy rainfall is usually accompanied by high humidity and low temperature, although the rainfall in this area is rarely much in defect, so the monsoon saturation deficiency varies very little from year to year, as shown in the top curve of Chart 4 (*a*), and the monsoon temperaure also varies far less than in the Punjab, for example, as shown in the second curve from the top in Chart 4 (*b*). Owing to uneven distribution of the monsoon rain, the years of the highest falls do not necessarily show very high humidity, for in both the wettest years of 1912 and 1914, with 71·86 and 86·88 respectively, over 40 inches fell in July alone and the saturation deficiency of the four monsoon months showed very little fall below the average, explaining once more why the humidity records are of greater importance than the rainfall data in relation to plague incidence.

There only remains the rainfall of October to December, shown by dark shading at the top of the columns, and this has an important influence on the humidity at this season, when plague reaches its maximum, and the saturation deficiencies show much wider variations than during the monsoon, as shown in the second curve from the top in Chart 4 (*a*). The data show that in the three years of excessive rainfall in 1902, 1916 and 1917 the saturation deficiencies were exceptionally low, and that in 1899, 1907, 1908 and 1923 exceptionally low rainfall was accompanied by high saturation deficiencies. Further, minor variations from this rule are almost all explained by temperatures above the normal, increasing the saturation deficiency and vice versa, so the relationship is almost constant, and high rainfall in the last quarter of the year, especially if associated with low temperature, as is commonly the case, is favourable to high plague incidence in the South Deccan.

Temperature.—The yearly variations in the hot weather temperature from March to May, and that of the monsoon period of July to September, are shown in Chart 4 (*b*), and the latter is too low to inhibit plague, and it varies too little from year to year to influence the disease materially, never having reached the major degrees indicated by — — or + + in Table V of the South Deccan climatic factors. The hot weather temperature varies rather more, but only once reached the major degree, and the mean monthly temperature at this season only varied between 79° and 81·5° F., so this factor also has comparatively little influence on the yearly plague incidence, especially as compared with the Punjab.

Saturation Deficiencies.—These factors remain as the most important in this area, although the monsoon one does not vary greatly from year to year. The highest saturation deficiencies are met with in the hot weather, as elsewhere, but the average figure is only about 0·500, or much lower than in Northern and Central India, but sufficiently high to bring about the annual hot weather fall of plague in the Deccan. The October to December figure averages just under 0·300, and the yearly variations are considerable in degree, so this is a most important climatic factor, occurring as it does at the height of the plague season. The South Deccan, therefore, presents very favourable conditions for studying the effect of varying saturation deficiencies on the yearly plague incidence, as the temperature conditions exert far less effect than in the areas so far dealt with, so the data in Chart 4 and Table V may now be analysed in detail.

Analysis of the Yearly Climatic and Plague Variations in the South Deccan.—The outstanding feature of plague in this area will be seen from Chart 4 to be the high prevalence from 1901–02 to 1904–05, and the recrudescences in 1911–12 and from 1915–16 to 1917–18. The periods are taken from June of the first year to May of the second, to cover each seasonal rise. The Southern Deccan was first affected by plague from Bombay as early as 1897–98, and in 1898–99 the mortality increased greatly from 0·40 in the previous period to 17·36 per mille, with favouring low temperatures in both periods and very low saturation deficiencies from March to December, and no unfavourable factors. In 1899–1900 there was a considerable decline to 7·45 per mille, owing to a slight excess of plague up to November, due to favouring hot weather and monsoon climatic factors being changed into low rates for the rest of the period, in accordance with very high and unfavourable saturation deficiencies for the two periods from October to February inclusive. In 1900–01 a further decline to 3·21 per mille took place, in relation once more to major degrees of high saturation deficiencies throughout the main plague season from October to February, moderately low temperatures from March to October producing little apparent effect on the disease.

A great rise to 26·98 took place in 1901–02 as the beginning of four very bad years, and this increase was associated with five of the six climatic factors

Table V.—Climatic Factors influencing Plague Incidence in the South Deccan (Bombay).

Year.	Data of first year.				Data of second year	
	Saturation deficiencies.	Temperatures.			Saturation deficiencies	
	March–May	March–May.	July–October	July–September	October–December	January–February
	Years of Increasing Plague					
6.98–5.99	− −			−	−	
6.01–5.02	− −					
6.11–5.12	+					
6.15–5.16	−		+		−	−
	Years of Continued High Plague.					
6.02–5.03	+	+			− −	− −
6.03–5.04	+ +	+				
6.16–5.17	+	+			− −	− −
6.17–5.18				−	−	−
	Years of Decreasing Plague					
6.99–5.00	−	− −	−		+ +	+ +
6.00–5.01	+	−	−	−	+ +	+ +
6.04–5.05					+ +	+
6.05–5.06	− −	−	+		+	
6.12–5.13	+					+
6.18–5.19		−		+	+	+
	Years of Continued Low Plague					
6.06–5.07	+ +	+				
6.07–5.08	+ +	+			+	
6.08–5.09	+				+	+
6.09–5.10	+				+	+
6.10–5.11	+	+				+
6.13–5.14	+				+	−
6.14–5.15		−				−
6.19–5.20	+ +	+			−	
6.20–5.21	+				+ +	+ +
6.21–5.22	+	+				+ +
0.22–5.23	+				+	−

being favourable to the disease. The rate of 1902–03 was almost the same, namely, 26·27, in spite of the incidence having been comparatively low in June and July in relation to unfavourable temperature and humidity in the hot season, for subsequently the rates were high in association with major degrees of low saturation deficiency in the two periods from October to February inclusive, the monthly incidence being once more in accordance with the particular seasonal variations of the climatic factors. In 1903–04 plague increased still further to the maximum rate of 34·83 per mille, in spite of unfavourable saturation deficiency in the hot season and moderately unfavourable high temperature in the monsoon period, so in this year the climatic factors failed to indicate the course of the disease.

In 1904–05 a considerable fall to 21·02 per mille was seen, due solely to high rates up to November, then undergoing a great decrease in association with a major degree of high saturation deficiency in October to December and a minor one in January and February. 1905–06 showed a further great decline to only 2·96 per mille, and this year showed moderate plague rates from August to November, in relation to favouring hot weather climatic condition, but little plague during the remainder of the period with unfavourable high monsoon temperature and high saturation deficiency in October to December.

During the next five years plague remained at a low level, and it is noteworthy that in every one of these years the climatic factors were predominatingly unfavourable to plague, especially the important saturation deficiencies.

In 1911–12 a remarkable rise of the mortality to 22·51 per mille was recorded, due mainly to the terribly high incidence of 37·58 per mille in the Dharwar district with infection of no less than 56 per cent. of the villages, and 61 per cent. of the whole of the plague of the whole of the Bombay Presidency took place in the three South Deccan districts, indicating some local conditions favourable to the spread of the disease, such as a special pilgrimage, for the climatic conditions showed only a minor excess of saturation deficiency in the hot weather and no factors favouring the disease The annual sanitary reports for Bombay throw no light on the cause of this sudden epidemic, which caused high death rates from June, 1911, to January, 1912, so it remains unexplained. In 1912–13 an equally rapid decline to 3·44 occurred with minor degrees of unfavourable saturation deficiencies in March to May and in January and February, and moderately favourable ones in October to December, and the rates remained low during the next two years with only minor climatic variations from the normal.

In 1915–16 a rise took place from 2·19 in the previous period to 6·96, with slightly high temperature in the monsoon period, but favourable low saturation deficiencies in the other three seasons of the year, and especially from October to December, when the main rise occurred. The rate remained high at 9·06 in 1916–17 with minor degrees of unfavourable conditions in the hot season, but favouring major degrees of low saturation deficiency from October to February, when the disease was at its height, and 1917-18 again showed a high incidence, 8·95, with four favouring minor degrees of climatic variation, and no opposing ones.

The last five years of the chart show low and slightly varying plague incidence, and it will suffice to point out that these years of continued low prevalence all showed predominating minor degrees of high temperature and saturation deficiency unfavourable to plague.

Summary.—With the exception of the unexplained epidemic of 1911-12, mainly in one district, the yearly variations in the incidence of plague in the South Deccan are dependent essentially on those of the humidity, as shown by the saturation deficiencies, for the minor differences in the temperatures in this area—comparatively cool hot weathers and monsoon periods—have little effect on the seasonal incidence of the disease. When the yearly variations in the humidity are considered in relation to the plague incidence in each season, the effect of high humidity in increasing and of low humidity in decreasing the prevalence of the disease appears to be established beyond doubt in this as in the other areas of India already dealt with. As, however, the most important climatic factors in the South Deccan are the saturation deficiencies from July to February, and especially from October to February, that is, during the yearly increase of plague, the meteorological data in this area do not allow the epidemic rises to be foreseen to nearly the same degree as in North and Central India, with much later seasonal increase of the disease and more effective variations in the hot weather and monsoon temperatures.

The plague and climatic variations in the North Deccan so closely resemble those of the South Deccan that it is unnecessary to discuss them in detail.

The plague incidence shown in Chart I is so low in Bengal, Madras and Burma, and the disease is almost absent from Assam, that the yearly variations in these areas do not furnish suitable data for similar studies, and in the absence of serious outbreaks forecasts would be of little value in them.

Conclusions.

1. The seasonal incidence of plague mortality in different areas of India can be explained by the seasonal variations in the mean temperatures and humidities, the latter being best expressed as saturation deficiencies, as pointed out by St. John Brooks. They appear to act through their influence on the life of the rat fleas, which carry the infection to man.

2. The yearly variations of plague incidence in the more severely affected areas of North-West, Central India, and the Deccan during the thirty years of

its prevalence in India have been studied in relationsip to the yearly variations of temperature and humidity with the following results :—

(*a*) The mean monthly temperature variations in the hot weather and monsoon periods influence the subsequent plague incidence by high temperatures reducing, and low ones favouring, the prevalence of the disease.

(*b*) The saturation deficiencies, both in the two hot seasons and in the early and late cold weather ones, influence the incidence of plague through high saturation deficiencies, indicating low relative humidity, being unfavourable to the prevalence of the disease, and vice versa.

(*c*) The great yearly variations in plague mortality can mostly be explained by studies of these climatic factors.

(*d*) In the three northern plague areas of the Punjab, the United Provinces and Bihar, four of the six seasonal climatic factors become evident before the regular annual rise of the disease from December onwards, and thus allow the more important yearly increases and decreases of the disease to be forecast to a large extent, and this is also the case in the Central Provinces, where the annual increase commences in the latter part of the monsoon period. In the Deccan area of Bombay, with an early monsoon increase of plague, and but slight yearly hot season temperature variations, the yearly variations in plague are more dependent on the saturation deficiencies during the plague season, so forecasts are of less value.

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Chart 1.

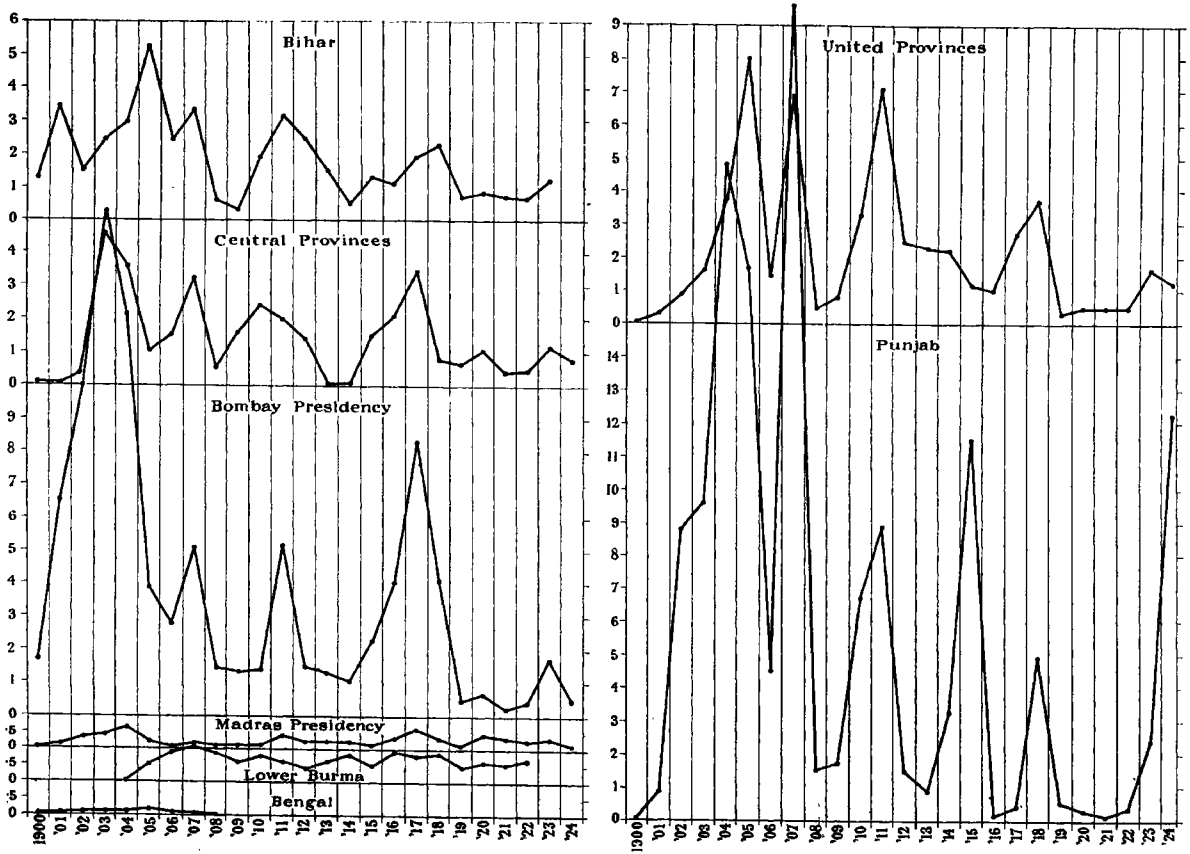

Bihar
Central Provinces
Bombay Presidency
Madras Presidency
Lower Burma
Bengal
United Provinces
Punjab
6
5
4
3
2
1
0
4
3
2
1
0
9
8
7
6
5
4
3
2
1
0
·5
0
·5
0
·5
0
9
8
7
6
5
4
3
2
1
0
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1900
'01
'02
'03
'04
'05
'06
'07
'08
'09
'10
'11
'12
'13
'14
'15
'16
'17
'18
'19
'20
'21
'22
'23
'24

Chart 2.

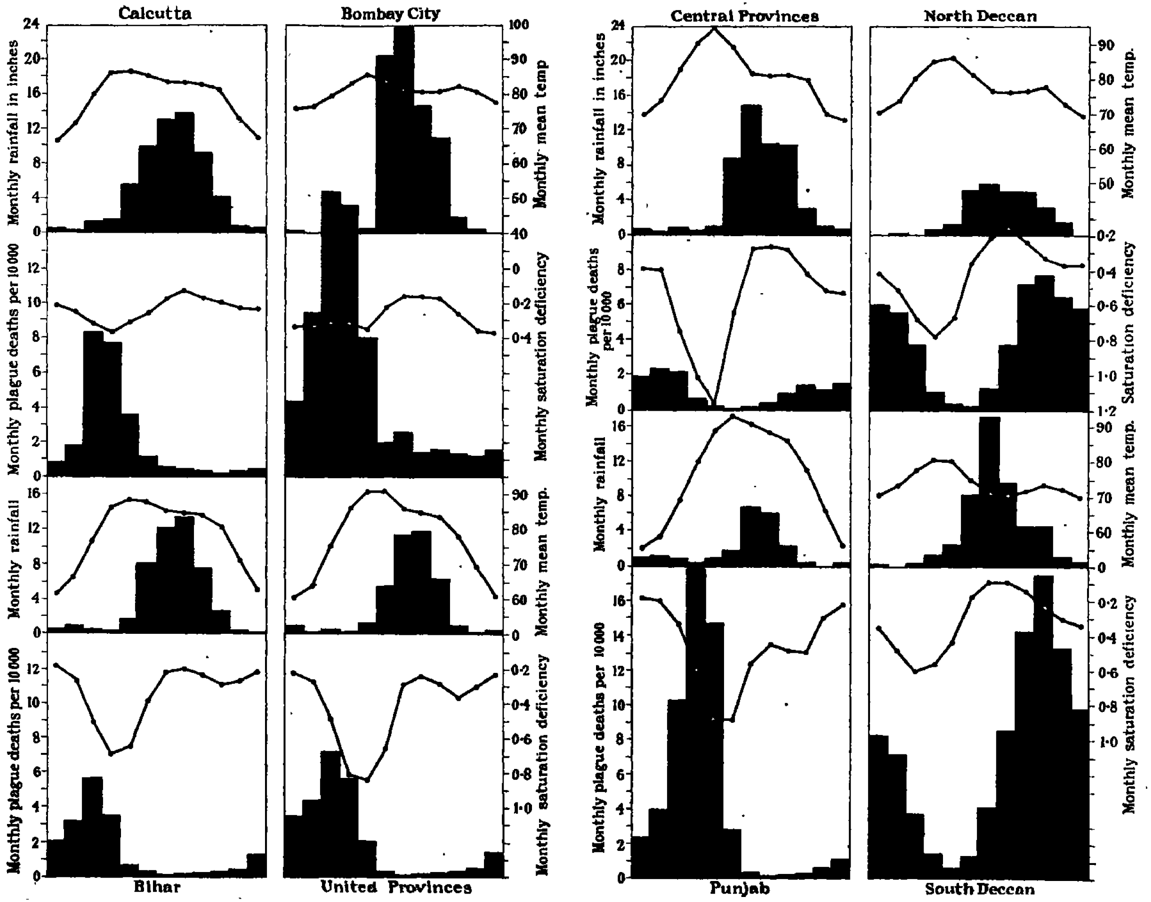
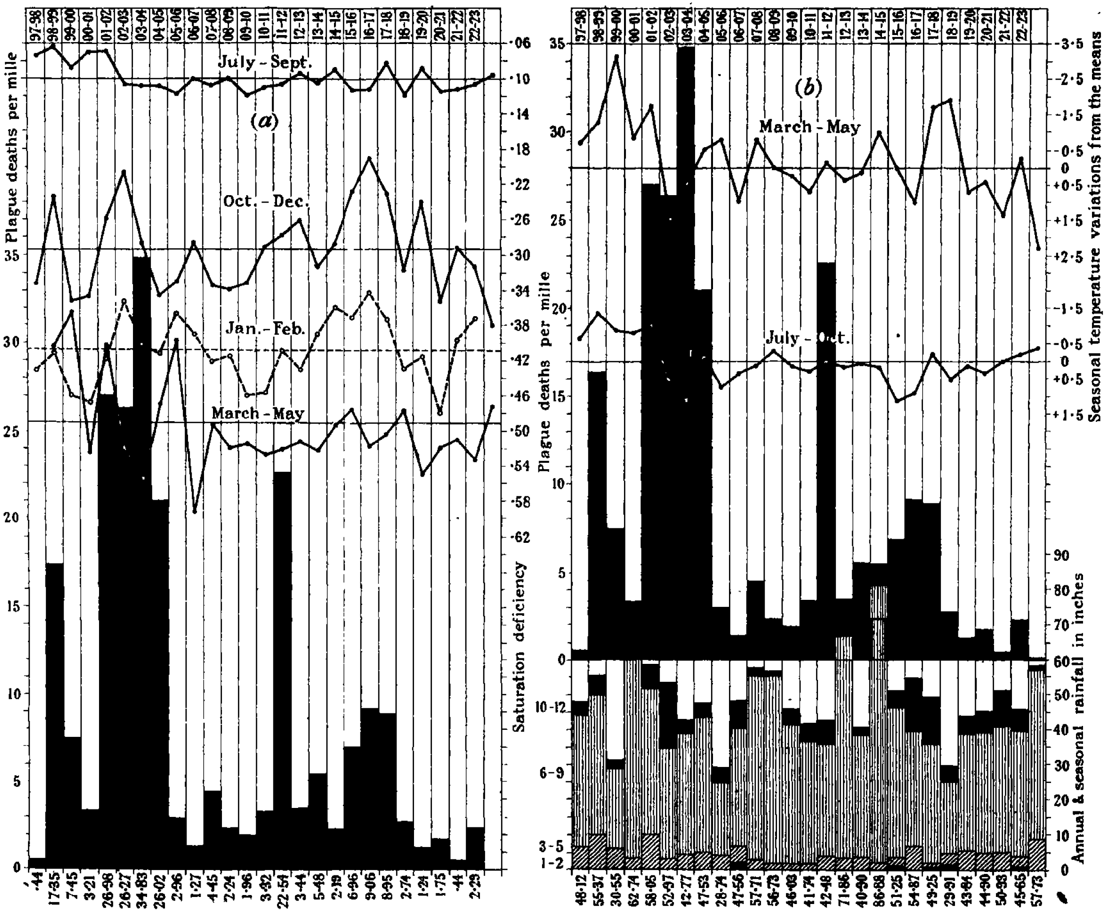
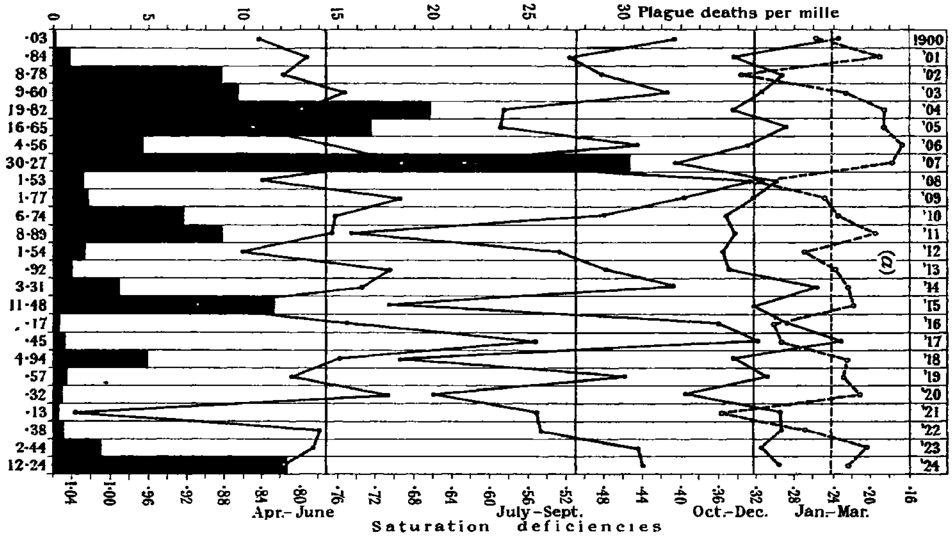
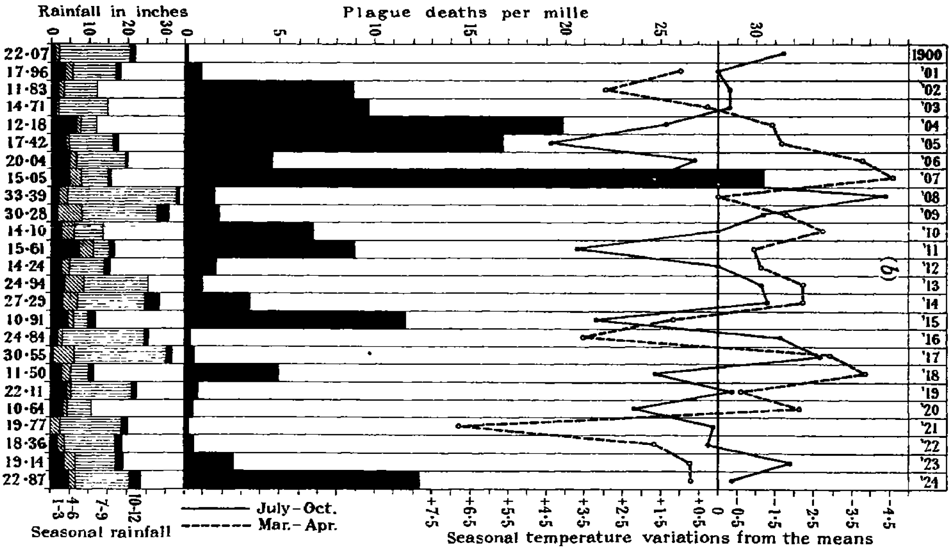

Calcutta
Bombay City
Central Provinces
North Deccan
Bihar
United Provinces
Punjab
South Deccan
Monthly rainfall in inches
Monthly mean temp
Monthly plague deaths per 10 000
Monthly saturation deficiency
Monthly rainfall
Monthly mean temp.
Monthly plague deaths per 10 000
Saturation deficiency
24
20
16
12
8
4
0
100
90
80
70
60
50
40
0·2
0·4
0·6
0·8
1·0
1·2
14

Chart 4.


(a)
Plague deaths per mille
35
30
25
20
15
10
5
0
Saturation deficiency
·06
·10
·14
·18
·22
·26
·30
·34
·38
·42
·46
·50
·54
·58
·62
July - Sept.
Oct. - Dec.
Jan.-Feb.
March - May
97-98
98-99
99-00
00-01
01-02
02-03
03-04
04-05
05-06
06-07
07-08
08-09
09-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
17-18
18-19
19-20
20-21
21-22
22-23
·44
17·35
7·45
3·21
26·98
26·27
34·83
26·02
2·96
1·27
4·45
2·24
1·96
3·82
22·54
3·44
5·48
2·19
6·96
9·06
8·95
2·74
1·24
1·75
·44
2·23
(b)
March - May
July - Oct.
Plague deaths per mille
35
30
25
20
15
10
5
0
10-12
6-9
3-5
1-2
Seasonal temperature variations from the means
-3·5
-2·5
-1·5
-0·5
0
+0·5
+1·5
+2·5
-1·5
-0·5
0
+0·5
+1·5
Annual & seasonal rainfall in inches
90
80
70
60
50
40
30
20
10
0
48·12
55·37
30·55
62·74
58·05
52·97
12·77
47·53
28·74
47·56
57·71
56·73
46·03
41·74
42·48
71·86
40·90
86·88
51·25
54·87
49·25
29·91
43·64
44·90
50·93
45·65
57·73


Plague deaths per mille
0
5
10
15
20
25
30
1900
'01
'02
'03
'04
'05
'06
'07
'08
'09
'10
'11
'12
'13
'14
'15
'16
'17
'18
'19
'20
'21
'22
'23
'24
·03
·84
8·78
9·60
19·82
16·65
4·56
30·27
1·53
1·77
6·74
8·89
1·54
·92
3·31
11·48
·17
·45
4·94
·57
·32
·13
·38
2·44
12·24
(a)
1·04
1·00
·96
·92
·88
·84
·80
·76
·72
·68
·64
·60
·56
·52
·48
·44
·40
·36
·32
·28
·24
·20
·16
Apr.-June
July-Sept.
Oct.-Dec.
Jan.-Mar.
Saturation deficiencies


Rainfall in inches
0
10
20
30
Plague deaths per mille
0
5
10
15
20
25
30
1900
'01
'02
'03
'04
'05
'06
'07
'08
'09
'10
'11
'12
'13
'14
'15
'16
'17
'18
'19
'20
'21
'22
'23
'24
22·07
17·96
11·83
14·71
12·18
17·42
20·04
15·05
33·39
30·28
14·10
15·61
14·24
24·94
27·29
10·91
24·84
30·55
11·50
22·11
10·64
19·77
18·36
19·14
22·87
(b)
1-3
4-6
7-9
10-12
Seasonal rainfall
July-Oct.
Mar.-Apr.
+7·5
+6·5
+5·5
+4·5
+3·5
+2·5
+1·5
+0·5
0
-0·5
-1·5
-2·5
-3·5
-4·5
Seasonal temperature variations from the means

Studies on the Relation of Gonadic Structure to Plumage Characterisation in the Domestic Fowl. IV.—*Gonad Cross-Transplantation in Leghorn and Campine.*

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Introduction.

Among the modern breeds of the domestic fowl it is possible to distinguish two types with regard to plumage characterisation. There are those breeds in which a typical dimorphism of the plumage in the two sexes is present. In some breeds this dimorphism is referable to regional differences in the shape, structure and colour of the feathers in male and female ; in other breeds although shape and structure of the feathers are different the colour is the same.

On the other hand, in the Sebright bantam and the hen-feathered Campine the plumage in both sexes is of the same type, being similar to that of the hen of those breeds in which sex-dimorphism of the plumage normally exists. The Campine is of particular interest for the reason that the breed includes two distinct strains, one showing a typical sex-dimorphism in respect to plumage whereas in the other the plumage of the male and female is similar in colour and structure.

That some definite relation normally exists between the reproductive organs and the type of plumage exhibited is shown by the change from hen-feathering to cock-feathering when either Sebright or hen-feathered Campine males are castrated, but it has not been shown that any morphological difference exists which could account for differences in physiological effect between the testis of the Sebright bantam, and that of the Leghorn. To determine whether there is any specific difference in the physiological activity of the testicular material of the two types of males, Roxas (1926) has attacked the question experimentally by gonad cross-transplantation between the Sebright and the Leghorn. The results of his experimentation were such as to permit him to conclude that " the growth of the head furnishings and the appearance of the sex instincts in the fowl are probably under the influence of the testicular secretions, while the plumage is conditioned by another factor or set of factors, presumably genetic, in addition to the testis."

Since it was found in the experiments of Roxas extremely difficult to obtain successful implants of Sebright testes into Leghorns (3 cases only, and even of these there was one in which the graft was not sufficiently active to effect the growth of head furnishings) the following cases from a series of birds, operated on during 1925–1927, are of interest in that they present additional confirmatory evidence of the effects on the plumage of such grafts from hen-feathered males to Leghorns. Furthermore, the principle derived from the experiments with Sebright testis, and applied by inference to other hen-feathered breeds, is shown to hold good when hen-feathered Campine males are used as a source of testicular substance for grafting.

Material and Method.

Two classes of experiment were performed. Class I—Implantation of testis from hen-feathered Campines to castrated Brown Leghorns; and Class 2—Implantation of testis from a White Leghorn to a castrated hen-feathered Silver Campine.*

Class 1.—The birds used were pure-bred Brown Leghorns and hen-feathered Silver Campine males. All the operations were performed between the seventh and the fourteenth day after the chicks were hatched.

An incision was made on the left side of a Brown Leghorn chick, under anæsthesia and the testis removed. A whole testis from a Campine chick of the same age was then implanted between the anterior lobe of the kidney and the posterior costa. No attempt was made to localise the graft other than by pressing it down into the cavity with a blunt instrument. After suturing the skin incision the chick was then turned over and a similar operation performed from the right side.

The birds were kept under observation for a period of from 16 to 22 months before they were killed and a *post-mortem* examination of the body cavity made to determine whether or not the operations had been successful.

According to the observations made it was possible to classify the birds into three groups :—

(*a*) Capons. The testes of the host had been completely removed and the implanted testes had not survived. It was possible to distinguish this group of birds by the diminutive size of the head furnishings at maturity.

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* It is well known that recessive cock-feathered ♂ ♂ are sometimes thrown by Campines, but in the strain made use of in these experiments over 200 birds have been bred without the appearance of such a bird. On this evidence it has been assumed that any cockerel chick used was potentially hen-feathered, although it was operated on before its definite type of plumage was declared.

(*b*) Birds in which castration had not been complete and in which nodules of regenerated testicular tissue were found. In some of these cases the grafted testes had survived, but in others no evidence of the persistence of the grafts could be found.

(*c*) Birds in which castration had been complete and the only testicular tissue present had been derived from the grafted testes.

For the purpose of the present discussion it is necessary to consider only those cases (four in number) belonging to this last group. In these there could be no reasonable doubt that the only testicular tissue present in the body of the bird at the time of the *post-mortem* examination was that derived from the implanted testes.

It was impossible to determine from an examination of the head furnishings before death whether a bird belonged to Group (*b*) or (*c*).

Class 2.—There is only one experimental bird in this class. Although the bird belongs to another series of experiments, the primary object of which was directed to the study of the growth of the head furnishings resulting from successful implantation of testis in a capon, the fact that the donor of the graft belonged to a breed in which there is a marked sex-dimorphism while the host was a hen-feathered Campine renders it possible to include it in such a study on the plumage characterisation.

Description of Cases.

1. Brown Leghorn, male—hatched May, 1925—operated on when 12 days old. Both testes were removed and four testes from two Silver Campine chicks of the same age were implanted, two on each side, anterior to the kidney.

When the adult plumage was attained it was seen to be typical of the normal Brown Leghorn Cock. There were no feathers simulating in colour, structure or shape those of the Brown Leghorn hen. The head furnishings increased in size, becoming red and turgescent, until they were equivalent in size to those of the normal cock of the breed. Actual comb measurements were not taken but during the whole period of observation the head furnishings were not observed to differ materially from those of normal cocks. The bird was under observation for 22 months and during that time the new feathers replacing lost ones were always like those of the cock of the breed. The bird exhibited throughout life sex behaviour and voice characteristics of the male.

On February 2, 1927, it was found to be badly infected with roup and was killed. Examination of the body cavity *post-mortem* showed that the castration had been complete. On the left side, projecting anteriorly from between the anterior end of the kidney and the body wall there was a large, somewhat lobulated graft, attached posteriorly to which were two thin-walled distended vesicles; another graft was present on the right side of the body anterior to and practically overlapped by the kidney The grafts were fixed in Allen's Modification of Bouin for histological examination.

Histology of the Grafts.—The left graft was found to consist of large testicular tubules with restricted intertubular spaces. Many of the tubules were apparently normal and in active spermatogenesis. In these, spermatogonial mitoses were frequently seen and there was no evidence of degeneration. In some of the tubules, however, degenerative changes were marked; these consisted chiefly of the sloughing off of the germinal cells into the lumen of the tubule which was filled with a mass of spermatozoa and cells earlier in the meiotic phase. The basement membrane was lined by a double layer of cells, a few of which were entering into the prophase of the first meiotic division. Scattered through the graft completely atrophic tubules were noted. In these the basement membrane was lined by a single layer of epithelial cells in which the cytoplasm formed strands stretching across the lumen, while the nuclei had their chromatin apparently concentrated into one or two nucleoli. There was no evidence of the onset of meiotic activity in these cells.

The right testis graft was enclosed by a much thickened fibrous capsule. The tubules were packed closely together and the intertubular spaces were correspondingly small The tubules varied greatly in size, some were large and others small, compressed and irregular in outline. In contrast to the graft from the left side of the body this graft showed that all the tubules were more or less undergoing degenerative changes. No evidence of spermatogonal activity could be found and the number of cells entering the meiotic phase was much reduced. The tubules frequently contained a mass of cells and sperm, the orientation of the different phases of the meiotic cycle to the basement membrane however was lost. (In normal tubules in spermatogenesis the cells entering the meiotic phase are near to the basement membrane. They are pushed further in towards the centre of the tubule by successive generations of cells from the layer of spermatogonia, so that in a functioning tubule those cells further advanced in meiosis are found nearest to the centre of the tubule.) Scattered throughout the mass of cells are many obviously degenerate, as shown by the formation of multi-nucleated giant cells and cells with pycnotic nuclei. Luteal tissue could not be identified in the grafts.

2. Brown Leghorn, male—hatched June, 1926.—Both testes were removed

in the second week after hatching and silver Campine testes from a chick of the same age implanted between the anterior end of the kidney and the body wall, one on each side of the body. The bird was kept under observation until the 1st of November (16 months) at which time a moult had just been completed, and then killed. It was with respect to head furnishings, plumage characterisation and behaviour a typical Brown Leghorn Cock. From the time of the assumption of adult plumage only feathers characteristic of the normal male were grown There was no indication of hen-feathering at any time during its history.

The weight of this bird prior to killing was 72 ozs.

Post-mortem examination showed that castration had been complete. There was one piece of grafted testis on the left side of the body anterior to the kidney, weighing 3·27 grams. On the right side large pieces of grafted tissue were found anterior to the kidney. The weight of these three grafts totalled 6·88 grams. That the right graft was found in three pieces when a whole testis was implanted was probably due to the fact that in forcing the testis down between the body wall and the anterior end of the kidney with a probe the testis was damaged, resulting in the rupture of the tunica albuginea and fragmentation of the gonad.

Histology of the Grafts —All the grafts presented essentially the same histological appearance Many of the tubules were apparently normal and in active spermatogenesis. In these large numbers of mature spermatozoa were present. In addition there were large tubules in varying stages of degeneration Following the desquamation of the cells the germinal cells were restricted to a single layer lining the basement membrane of the tubule and the lumen was filled with masses of mature sperm and cell débris. A few characteristic atrophic tubules were present. In none of the grafts were definite interstitial elements identified.

3. Brown Leghorn, male—hatched May, 1926—operated upon during the second week after hatching. Both testes were removed and the two testes from a Silver Campine chick of the same age were implanted, one on each side of the body between the anterior end of the kidney and the body wall.

The bird was killed on November 8, 1927, at the completion of a moult. Never during the time it was under observation subsequent to the assumption of adult plumage had it produced any feathers not typical in colour, structure and shape of the Brown Leghorn cock. The behaviour during life was typically male. At sexual maturity the bird possessed the large vascular head furnishings of the cock and these were retained throughout its life. The weight of the bird when killed was 69 ozs. Examination *post-mortem* showed that castration had been complete and that successful implantation of testis had been achieved. On the left side of the body there was a large testis graft where the original implantation was made behind the anterior lobe of the kidney. On the right side of the body two grafts were present attached to the body wall and in front of the right adrenal gland.

Histology of the Grafts.—The three grafts presented histologically the same appearance—there were many apparently normal tubules in active spermatogenesis. Deviations from the normal were found in some of the tubules in which cells in varying phases of the meiotic cycle had lost their orientation with regard to one another and their position in the tubule. As a result cells in all phases of activity were indiscriminately mixed together. In other tubules the only deviation from the normal appeared to be the reduced number of cells in the meiotic phase. The small graft from the right side of the body was furthest from a normal condition of the seminiferous tubules. There was no evidence of luteal tissue present in the grafts.

4. Brown Leghorn, male—hatched May, 1926—operated upon during the second week after hatching. Both testes were removed and Silver Campine testes from a chick of the same age implanted between the anterior end of the kidney and the body wall, one on the right side of the body and one on the left.

The bird was killed on November 8, 1927, at the completion of a moult. It had never assumed any features suggestive of female characteristics while under observation. From the attainment of sexual maturity until the time of death, the cock possessed the large vascular head furnishings of a normal male of the breed. Its weight at death was 65 ozs.

Histology of the Grafts.—Both grafts contained seminiferous tubules of various sizes, the largest of which were in active spermatogenesis and comparable to active tubules in a normal testis. In a few of them the different nuclear phases in meiosis were indiscriminately mixed, but otherwise there was no indication of cellular degeneration in these tubules. The smaller tubules showed a much restricted spermatogenic activity, having only comparatively few cells entering the meiotic phase and an almost complete absence of ripe spermatozoa. The seminiferous tubules in the grafts were packed closely together and the intertubular spaces small. No luteal tissue could be identified in the grafts.

5. Hen-feathered Silver Campine, male—hatched 1925—castrated in the first week of life. When the bird reached maturity it possessed the typical appearance of the completely gonadless bird. The comb and wattles were small and pale, the plumage was of the cocky type but longer and more luxuriant. (Morgan (1915) has shown that castration of males of the "Hen-feathered"

breeds results in the production of a type of plumage characteristic of the males of the "cock-feathered" breeds.)

On May 10, 1927, two years after the removal of the testes, the bird was operated upon and one testis from a White Leghorn chick, five weeks old, was implanted subcutaneously on the underside of the left wing The comb volume at the time of operation was 1 c.c.

Five weeks after the operation the face and head furnishings were noticeably pink, and at this time certain areas of feathers were removed from various regions of the body and the denuded areas outlined by tattooing with Indian ink. The comb and wattles began to enlarge and the comb reached a maximum volume of 8 c.c. 12 weeks after the implantation of the graft. It then steadily declined, reaching a minimum level of 2 c.c. 23 weeks after the operation. The new feathers in the plucked areas were removed when full grown and were followed by another feather generation. Thus, during the course of the experiment, three lots of feathers were obtained, the first on June 16, the second on September 20, and the last on December 9. The feathers plucked on September 20 had grown during the period when the comb was developing to a maximum and through the following period when the comb regressed Those plucked on December 9 underwent the greater part of their growth when the comb had regressed until it reached a minimum stable level. None of the feather generations showed any characteristic that could be taken as an indication of a change in the type of feathering from a cocky to a henny structure.

Twenty-six weeks after the original implantation the graft was removed, and there was no subsequent reduction in the size of the head furnishings which remained constant with a volume of 2 c.c The weight of the testis graft after removal was 0·21 grams. During the growth of the comb the bird was observed to crow vigorously.

Histology of the Grafts. -The graft was enclosed by a thickened capsule of fibrous tissue The seminiferous tubules varied considerably in size and activity Many were atrophic and in these the cells were restricted to a single layer lining the basement membrane of the tubule. Many of the larger tubules contained cells representing the varying phases of the maturation phenomena up to and including ripe spermatozoa. Although in these tubules some spermatogonial mitoses and first meiotic division stages were met with, the central lumen was filled with cellular débris in which were found multinucleated giant cells, mature spermatozoa and other cells in the advanced stages of meiosis. None of the tubules had the appearance of normal seminiferous tubules in active spermatogenesis. At various points under the fibrous sheath of the gonad there were relatively enormous accumulations of lymphocytes. Careful examination failed to reveal the presence of any luteal tissue in the graft.

Conclusions.

It is seen that in the case of Brown Leghorn males from which the testes have been removed, the successful implantation of testicular tissue from hen-feathered Campines in no way modified the development of the male characters normal to a Brown Leghorn cock. The enlarged head furnishings, the behaviour and voice all developed exactly as in the normal Leghorn cock and so remained during the whole time (16-22 months) the birds were under observation. The plumage assumed at maturity was that of the typical male Brown Leghorn, and this was retained through successive feather generations up to the conclusion of the experiments. There was no evidence at any time of structural modifications of the feathers in the direction of the female type.

Since an amount of Campine testis sufficient to induce full expression of the male comb, behaviour and voice in castrated Brown Leghorns did not produce a transformation of cocky into henny plumage, these results support the conclusion of Roxas that hen-feathering in the male of the hen-feathered strain of Campines cannot be due to an endocrine difference between the testis of the two breeds.

On the other hand, the subcutaneous implantation of a testis from a Leghorn male into a completely castrated hen-feathered Campine male, although it caused increased vascularity and growth of the head furnishings, did not induce any structural modification of the feathers during the course of the experiment The bird did not assume the henny-feathering typical of the cock of this strain The hen-feathered male Campine, when castrated, becomes typically cock-feathered. On the basis of the experiments of Roxas, in such a bird with a functioning testis graft from a Leghorn it would be expected that new feathers grown while under the influence of the graft would be structurally female.

It is interesting to note that Roxas described a case of a Sebright with Brown Leghorn testis successfully implanted, which, although effecting an increase in the size of the head furnishings, was only responsible for a partial change in the feathering towards the female type. In the other cases reported by him, the change from the cock-feathering of the capon to hen-feathering was complete. In the Campine under consideration the presence of the testicular graft did not lead to the full development of the head furnishings, the maximum size attained being only about one-third that present in the normal male at maturity. It has been stated by Morgan that a smaller amount of testis suffices to keep the comb at or near its normal size than to keep the feathers true to type for hen-feathering. If this be so, then, in the bird under discussion one would expect that since the graft was not sufficient to effect a maximum development of the comb, it was still less likely to cause a change in the character of the plumage from male to female.

Summary.

1. Four cases of successful implantation of testicular material from the hen-feathered strain of Campines into castrated Brown Leghorn males are described.
2. The grafts although producing the normal male head furnishings and behaviour did not lead to a change in the plumage character from *cock* to *hen* feathering.
3. These results are in accordance with those obtained by Roxas when testis from Sebright bantams was implanted into castrated Brown Leghorns and support his conclusion that the "hen-feathering" of the male in the hen-feathered breeds cannot be due to an endocrine difference between the two kinds of testes.
4. Successful implantation of a testis from a Leghorn male into a castrated hen-feathered Campine did not result in a change in the plumage from cock to hen-feathering as would be expected on the basis of the above hypothesis.
5. It is suggested that failure on the part of the graft to change the type of feathering was due to an insufficient amount of testis, since it was incapable of inducing a normal development of the head furnishings.

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The Agricultural Value of Rainfall in the Tropics.

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I.—*Introduction.*

The objective of the investigation which is here described was, in the first instance, economic. Having assumed responsibility for the official forecasts of the main crops of the United Provinces, India, it appeared to the writer that, in a country where rainfall so dominated the agricultural conditions, it should be possible to evolve some system, based upon rainfall data, of forecasting both area and yield of crops which would be free from dependence on the very doubtful personal equation involved in the methods then in force. The considerable measure of success achieved in forecasting areas led to an attempt to forecast yields—a much more difficult problem.

As the work proceeded the method assumed a wider significance bearing on the general problem of the availability of soil moisture for plant growth. In a year's growth, whether this be from a seed or a freshly planted slip, the yield, in whatever form it be measured, is the summation of the various reactions of the plant to its environment at every stage of its growth; in the case of annuals it may even, as Hooker (8) has suggested, include the reaction of the parent plant. What applies to the sum must, *a fortiori*, apply to the component parts; and it would appear, therefore, that the method employed to evaluate rainfall should afford a means of interpreting the physiological processes of the growing plant, in so far as these are dependent on rainfall.

Rainfall is not the only factor which enters into such a consideration, but, in the tropics, it would appear to be the dominant one, while, from the results obtained and here described, that dominance is such that, of the environmental factors, rainfall alone need be considered when forecasts are in preparation.

II.—*Methods.*

The relationship which exists between crop and rainfall has formed the basis of a considerable body of work; notably that of Hooker (8 and 9), Watt (24) and, more recently, Fisher (7). In America, the work of Warren Smith (22 and 23), Kincer (13 to 15), Moore (18), Conner (5), Wallace (20) and McDonald (17) is directed to the same end, while under conditions more nearly approximating to those dealt with below, the work of Jacob (10 and 11) in India and

Walter (21) in Mauritius bears on the same problem. With the exception of Fisher, all the above writers rely for the evaluation of rainfall on the summation of the fall for equal intervals of time, the intervals in individual cases varying from 8 weeks to 7 days, and for the calculation of the effect on yield on the regression equation involving the partial correlations.

This method, involving as it does the aggregate rainfall of a particular period, is open to criticism. Each unit of time is treated as independent and self-contained, whereas it is clearly not the case that the effect of rainfall is thus discontinuous. Anteriorly the effect of rainfall is discontinuous, since rain becomes effective only after the fall itself; posteriorly, however, the effect is continuous.

The method here adopted is designed to meet this characteristic of rainfall. A continuous " effective rainfall " curve is built up from the daily rainfall data, the value of the " effective rainfall " (E_n) of any particular day being calculated from the formula

$$E_n = r_n + kr_{n-1} + k^2r_{n-2} \ldots + k^{n-1}r_1,$$

where r_n is the rainfall of the 24 hours ending on the morning of the nth day and k is a constant having a value < 1.

This may be written

$$E_n = r_n + kE_{n-1},$$

and if, as in the case of prolonged drought, $r_2, r_3, \ldots r_n$ are all zero, the " effective rainfall " curve takes the form

$$y = r_1 \varepsilon^{-ax}$$

where $-a = \log_e k$.

The effective rainfall of any particular day thus includes not only the rainfall registered at 8 a.m. on that day but a carry over from the preceding day, which in its value takes into account both the amount and distribution of the antecedent rain. The constant k is a measure, though not an absolute measure, of that fraction of the rainfall which remains available for plant growth. The value is here taken to be 19/20.

It follows from the above that $(1 - k^{n-1})\, r_1$ is a measure of that fraction of the rainfall which ceases to be available for plant growth after n days, whether the cause be a loss through run-off, seepage, direct evaporation and transpiration, or whether the water be held by the soil in an " un-free " form. As a numerical illustration, it will be found that a rainfall of 10 inches in one day (a heavy but not unrecorded amount in the tropics) gives an effective rainfall of 0·47 inch on the 60th day; in other words, its effect becomes negligible during the third month.

Further discussion of the significance of the effective rainfall and the constant k is postponed till later.

III.—*The Data.*

Any one who attempts the solution of such a problem as the relation between rainfall and crops is faced at the outset with the difficulty of securing data of which the concordance is above suspicion. Since only one record per annum is possible under ordinary circumstances, direct experiment, having as object the collection of data required, places that collection beyond the capacity of the individual. Recourse has to be had to official returns, the records of private estates or those of experimental farms. Rarely, and especially in the tropics, are these entirely free from objection. In only the four series which follow did the reliability appear sufficient to justify the somewhat tedious arithmetical labour involved.

(1) The Unirrigated Wheat Area of the Cawnpore District, United Provinces, India.

(2) The Unirrigated Cotton Area of the Cawnpore District, United Provinces, India.

The former refer to a series of 29 years and the latter to a series of 17 years. These figures are admittedly very accurate, being employed for revenue purposes. The corresponding rainfall figures are taken from the daily rainfall data published by the Indian Meteorological Department. The value of the daily rainfall is taken at the average of the daily readings of the various recording stations situated in the District.

(3) The crop yield of a Barbados sugar estate covering a series of 17 years. Both the yields and the daily rainfall data were secured through the courtesy of the present proprietor and Mr. C. C. Skeete, Assistant Director of Agriculture, Barbados.

(4) The yield of cotton of the Dhulia Experiment Station, Bombay Presidency, India.

Both the yields and the daily rainfall data were secured through the courtesy of Dr. H. H. Mann, formerly Director of Agriculture, Bombay.

IV.—*Proof of the Significance of the Effective Rainfall.*

The first two of the above series are concerned with the moisture conditions of the soil at the time of sowing. They constitute, therefore, a test of the value of the effective rainfall figure as a measure of the agricultural value of rain.

(1) *The Unirrigated Wheat Area of the Cawnpore District.*—The monsoon rainfall usually ceases in September and wheat is sown at the commencement of the cold weather in late October or early November. It is sown, therefore, on a falling moisture; it is sown, too, on land left fallow and constantly cultivated throughout the monsoon. If, now, a tract such as the Cawnpore District be considered, with its divergences of soil, its higher and lower lands and other differences, it would appear that the area of a crop, dependent for its germination on the natural conditions of moisture, would be determined by the amount of accumulated rain held by the soil and available for plant growth This, in its turn, is dependent less on the total monsoon rainfall than on its distribution. The test of this supposition is supplied by the correlation found between the value of the effective rainfall at the end of the monsoon, the actual date selected being October 15, and the unirrigated wheat area

The figures obtained for the series of 29 years analysed are :- -

Correlation, unirrigated wheat area, Cawnpore District :—

Total monsoon rainfall .... $r = +0{\cdot}69 \pm 0{\cdot}06$

Effective rainfall, October 15 ... . $r = +0{\cdot}82 \pm 0{\cdot}04$

Using the regression equation derived from the latter figure, it has been found possible to forecast, some 15 days before sowing, the unirrigated wheat area with an accuracy which is greater than that of the official forecast, laboriously compiled from detailed returns and published some 3 months after sowing.

(2) *The Unirrigated Cotton Area of the Cawnpore District* - This analysis is less simple. In the first place, owing to the irregularity in the date of the break of the monsoon, the selection of the effective rainfall value by calendar date is open to objection. This objection, however, is minimised by the fact that the oncoming cold weather strictly limits the cotton season and sowing must be completed sufficiently early to enable the plant to mature its crop. Practical experience, fortified by numerous calculations, indicates that the critical date is July 7. In very late seasons cotton may be sown up to July 15 after which date little or no cotton will be sown whatever the rainfall conditions.

In the second place, cotton is not, like wheat, a basal crop of the District; in many respects, it is alternative to wheat. It is sown on similar land and the cultivator makes the decision whether he shall sow cotton or retain the land fallow for wheat later. Moreland (19) was the first to point out this relation, and he adopted, as a measure of the relative importance of wheat and cotton in the eyes of the cultivator, the cotton-wheat price ratio. He carried his figures back to a date prior to the American Civil War and, further, used the total cotton area of the Province. His conclusion, as it stands, is not supported by the figures, which give a correlation coefficient of only + 0·10 ± 0·10. Nevertheless his conclusion has a basis of truth.

It is in the case of *irrigated* cotton that the cultivator is influenced by this consideration. Using the figures for the irrigated cotton of the Cawnpore District for the 17 years here analysed, the correlation with the cotton-wheat price ratio is found to be + 0·53 ± 0·12. That a like consideration does not influence the cultivator in the case of unirrigated cotton is indicated by the corresponding correlation for the unirrigated area, which is − 0·06 ± 0·16. It would appear that the cultivator is governed by different motives in the case of unirrigated cotton. "A bird in the hand is worth two in the bush," and, with a favourable monsoon, he will sow cotton up to the limit; the later rains may be unfavourable for wheat, and, in any case, if the cotton fails, it can be ploughed up and wheat or barley sown. There is, therefore, no reason to suspect lack of concordance on these grounds in the present analysis.

Thirdly, and lastly, with a favourable monsoon, the maximum unirrigated area will be sown before July 15, and subsequent rain, even though it fall before that date, will not affect the area; in other words, the earlier the rainfall, the greater its potential value for sowing. The interval between June 5 and July 15, being respectively the earliest date of the break of the monsoon and the end of the sowing season, is 40 days. If it be assumed that a rainfall on the earlier date gives to the effective rainfall figure a value double its actual value, with proportionate increments to the values of the effective rainfall of intermediate dates, an allowance is made for the time factor by a system of weighting.

The following correlations show both the superiority of the effective rainfall as a measure of the value or rainfall for sowing purposes and a progressive efficiency with the successive allowances made:—

Correlation, unirrigated cotton area, Cawnpore District:—

Total rainfall to July 7 (18 years)	$r = +0{\cdot}47 \pm 0{\cdot}12$
Effective rainfall on July 7 (18 years)...	$r = +0{\cdot}61 \pm 0{\cdot}10$
Average daily effective rainfall (17 years), 1st total of 0·5 inch to July 7	$r = +0{\cdot}61 \pm 0{\cdot}10$
Average daily effective rainfall (17 years), 1st total of 0·5 inch to July 15 (weighted)	$r = +0{\cdot}77 \pm 0{\cdot}07$

There is little doubt that, as in the case of the wheat area, the use of the regression equation would result in a forecast of the unirrigated cotton area which would be more accurate than the present estimate. The two analyses here given would appear to indicate that the effective rainfall constitutes a significant measure of the agricultural value of rain, and is a measure which possesses distinct advantages.

V.—*The Effective Rainfall as a Determinant of Yield.*

The two later series deal with the yield, and here the problem is essentially one of the interpretation of natural phenomena, the physiological reaction of the plant to rainfall.

The majority of crops, as any practical agriculturist will testify, are subject to two evil influences traceable to moisture, there may be excess as well as deficiency. Between these two extremes there lies a more or less wide range of moisture conditions which may, other conditions being equal, be considered the optimum for the growth of the plant, and, provided the moisture content never rises above, or falls below, these limits, a full yield should result

(3) *Barbados Cane.* Barbadian agricultural practice is of such a high order and so standardised that there is no reason, on these grounds, for anticipating discordance in the records over a series of years. One source of discordance, however, cannot be disregarded; it arises out of the practice of the frequent introduction of new varieties. Fortunately, however, for the present purpose the change to a new variety is not made abruptly, and it is, therefore, possible to introduce a correction which minimises this source of discordance. In all cases the yield values, so corrected, give a better result, when judged by the correlation coefficient, than is given by the absolute yields

The cane crop occupies the ground for some 15 months. The harvest lasts from February till May and is coincident with the dry season. From the aspect of rainfall, the crop period may be divided into three sections; firstly, the planting period, when adequate moisture is required for the establishment by the sets of a root system which will enable the plant to resist the dry weather, secondly, a dry period, ending in May or June, and, lastly, the wet season lasting to December or January. The dry season is only relatively dry and its commencement and termination vary from year to year within very wide limits. It is not possible, therefore, to adopt, as in the case of wheat dealt with above, the figure of the effective rainfall of a particular day as a measure of the seasonal rainfall. Further, from the peculiar formation of Barbados as a coral island, there appears to be a phenomenon not commonly found. The spongy coral rock forms a reservoir from which water is returned to the surface to an extent far in excess of the normal. A similar condition is found in the sub-Himalayan tract of Bengal and Bihar, where, however, the upward thrust is derived from the accumulation of the monsoon rainfall in the hills (16). Consequently, the available moisture depends less on the effective rainfall than on the accumulated store of water held in the coral rock beneath, of which the effective rainfall of the previous wet season is found to be a better measure than the actual rainfall of that period.

The following are the results obtained :--

Correlation, corrected yield, Barbados cane :—

Aggregate rainfall of previous wet season (17 years) .... $r = + 0{\cdot}24 \pm 0{\cdot}15$

Average effective rainfall of previous wet season (16 years) .... $r = + 0{\cdot}44 \pm 0{\cdot}14$

During the dry season the plant adds little to its visible growth; what invisible growth of the root system takes place during this period is not known. The plant is, however, capable of responding to such rainfall as may occur.

Correlation, Barbados cane :—

Yield and average effective rainfall of dry season (December to May) (16 years) .... $r = + 0{\cdot}36 \pm 0{\cdot}15$

Corrected yield and average effective rainfall of dry season (December to May) (16 years) .. $r = + 0{\cdot}42 \pm 0{\cdot}14$

The main factor influencing final yield, however, is the rainfall of the wet season of the crop period, June to December.

Correlation, Barbados cane :—

Yield and average effective rainfall of wet season (June to December) (17 years) .... $r = + 0{\cdot}74 \pm 0{\cdot}07$

Corrected yield and average effective rainfall of wet season (June to December) (17 years).. $r = + 0{\cdot}76 \pm 0{\cdot}07$

Taking the figures for those 16 years only for which all three series are available, the coefficient of triple correlation is found to be :—

$$R = 0{\cdot}76.$$

It is possible, however, to approach the subject from a different angle. The rainfall of the wet season being found to be the most important determinant of yield, and yield, in a plant like cane, being a function of the growing season, the months of June and December, lying respectively at the commencement and end of the growing season, become critical months. It might be anticipated, therefore, that a high degree of correlation would be found between the yield and the aggregate effective rainfall of these two months. This correlation is found to be :--

$$r = +0{\cdot}67 \pm 0{\cdot}09.$$

All attempts to trace an effect injurious to yield of excessive moisture have led to negative results, and it is only in the lower limits that moisture exercises a controlling influence. General agricultural experience that cane is singularly resistent to excessive moisture is thus confirmed, and in Barbados the upper limit is rarely, if ever, attained.

(4) *Dhulia Cotton.*—There appears to be only one source of discordance here requiring note; it is the question of what is commonly termed "stand" During the early stages, and especially during germination, the plant is very sensitive and re-sowing has frequently to be employed Unfortunately, no record is available of the "stand" for the present series and no correction for it is possible.

The analysis of these returns offers a problem which is more complex than any of the three preceding examples Partly this is due to the morphology of the cotton plant itself, and partly to the fact that cotton is so sensitive to excess moisture that such excess is a normal occurrence under the conditions prevailing.

The morphology of the cotton plant is too well known to require detailed description. The apical bud grows indefinitely, while the secondary branches, with the exception of a few at the base of the stem which are monopodia, are all sympodia. The extent of flowering and, indirectly, of fruit production, therefore, is closely associated with growth. Of, perhaps, greater importance in the matter of yield is the number of flowers which set and develop into bolls Under normal conditions, as growth, with the accompanying flower production, proceeds, more and more of the food materials built up by the plant is absorbed by the developing fruits, with a consequent reduction of vegetative vigour, which, in its turn, limits the production of flowers. Where the vast majority of the fruits set growth will practically cease, and this will take place before the first formed fruits have opened and while they still exercise a drain on the food supply. As, however, the bolls open, more and more of the vigour of the plant is redirected to vegetative growth, with a further production of flowers borne on the upper secondaries and on the tertiaries arising from the lower monopodial secondaries. The normal flowering curve of the cotton plant is, in consequence, bimodal, the early mode being very sharply defined, and the total yield will be built up from the produce of the two flushes.

Such is the normal condition but where the season is short temperature or humidity may check growth to such an extent that the second flush may be absent. If, too, for any reason, and one of the most important of such is excessive rain, the shedding of flowers or young fruits assumes large proportions, this check to vegetative growth will not occur and, accompanying that continuous growth, will be continuous flower production. Again, the flowering curve will no longer show the bimodal form. Yield will here be built up gradually.

During early development and until a firm root system is established, the cotton plant is distinctly delicate, especially to conditions of excessive moisture. The limits between which lie suitable values of humidity are, therefore, at this stage, narrow. Subsequently these limits broaden during the vegetative period and only drought or excessive moisture over a prolonged period will affect vigour adversely. From about the 60th day, however, when vigorous flowering is in progress, a dry period is essential if fruit is to set. After a further period of 20 to 30 days, the rate of flowering diminishes, owing to the check administered to vegetative growth. The plant is now capable of withstanding a heavy spell of wet weather, and, in fact, requires a full supply of moisture if that vegetative growth is to take place which will result in a second flush. But this wet spell must be of limited duration or the early ripening fruits will be injured.

Based on these considerations, a pair of curves can now be built up which will represent the upper and lower limits of the moisture conditions, as measured by the effective rainfall, favourable to the production of a heavy yield. The sum of the divergences, both positive, above the maximum, and negative, below the minimum, should, if the argument be well founded, form a measure bearing a negative correlation with the yield

Actually, however, the cotton plant is not the rigid automaton so far conceived. It displays a certain amount of flexibility which exhibits itself in the following manner. Dividing the growth period into three sections, initial growth, first flowering period and subsequent growth, each period is capable of limited extension, depending on the climatic conditions, without a material effect upon yield. A dry initial period means reduced vegetative growth and may be prolonged. It appears to require an aggregate of at least 16 inches effective rainfall if a full first yield is to be obtained. A wet initial period may also be extended without injury to the yield, provided a dry period supervenes. Similarly the second period may be prolonged within limits, while the third period is only limited by the continued drought and falling temperature which follows the termination of the monsoon. The dual curve, in fact, takes the form of a concertina, the sides of which are not parallel, and of which the capacity to extension is limited to three points. These are marked ↑ in the accompanying diagram (p. 92).

An analysis of the Dhulia cotton crop carried out on these lines yields the following results :—

Correlation, cotton yield, Dhulia (16 years) :—

Aggregate rainfall of monsoon	$r = + 0{\cdot}41 \pm 0{\cdot}14$
Average effective rainfall of monsoon	$r = + 0{\cdot}47 \pm 0{\cdot}14$
Sum of divergences, whether positive or negative, from curves of limiting moisture	$r = - 0{\cdot}70 \pm 0{\cdot}10$

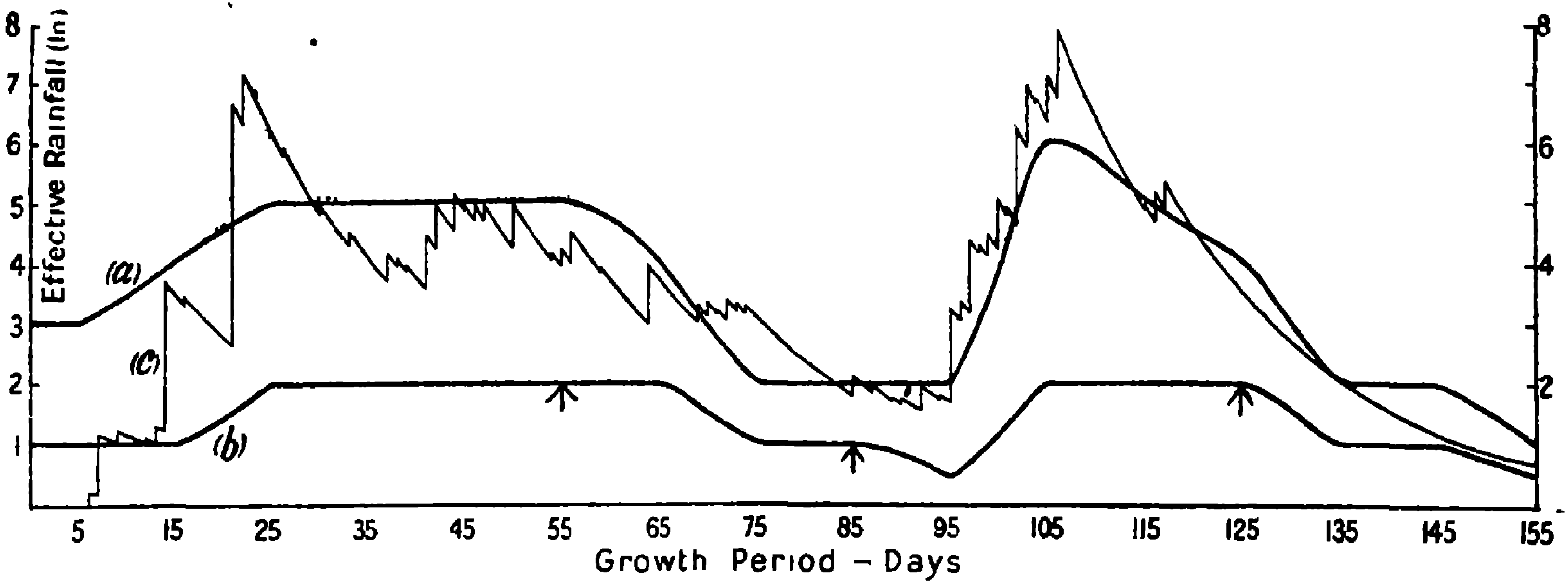
In the case of any series of records which have not been collected for the specific purpose for which they are employed and which are, therefore, lacking in certain pertinent particulars, the risk of attempting too much is a real one. For this reason it would be unprofitable to carry the analysis further in the present instance.

VI.—*Discussion.*

The measure of rainfall, to which the name " effective rainfall " is here given, is a measure which has considerable value in that it affords a means of estimating, and even of forecasting, data with regard to crops. The main interest, however, lies in another, and more theoretical, direction and centres around the interpretation to be placed on *k*, here adopted as a constant.

k being a measure of that fraction of the rainfall which becomes available for plant growth, it is only necessary to consider the destination of that fraction of the rainfall which fails to become available—run off and percolation, dependent on soil conditions , direct evaporation and transpiration, dependent on temperature and air humidity among other factors; the " unfree " water held by the soil, dependent on such soil characteristics as clay and humus content—to see that *k* cannot be a constant universally applicable.

(1) *Temperature.*—Both direct evaporation and transpiration are, to some degree, determined by temperature. The method takes no account of such variation beyond that implicit in the title of this paper. The value here adopted for *k* carries the limitation which the word " tropics " implies and is applicable only under those conditions in which temperature does not directly inhibit plant growth. Where, as in the temperate zone, a marked seasonal change from summer to winter, occurs, the problem becomes more complex. The



Dhulia Cotton, Bombay Presidency.—Limiting curves calculated to 10-day intervals.—(*a*) Maximum; (*b*) Minimum; (*c*) Effective rainfall curve, season 1914.

The diagram shows maximum (*a*) and minimum (*b*) effective Rainfall Curves for the short-season Indian Cotton. Superimposed is (*c*) the actual effective rainfall curve for the season 1914, at the Dhulia Experiment Station. The initial, vegetative, period is too short and too wet in the earlier stages, while the second, or first flowering, period is also too short for a full yield to result. The actual yield was 802 lbs. seed cotton, a full yield being 1000 to 1050 lbs.

direct effect of temperature on the plant becomes of importance while *k*, as a measure of loss of water, is no longer a constant but a function of temperature.

(2) *Air Humidity.*—Under the climatic conditions prevalent in a rains tract, there is a close association between rainfall and air humidity. The effect of air humidity as a separate independent factor may, therefore, be disregarded. The conditions, however, are very different in the case of irrigation. Here the relation between air humidity and water supplied is relatively small, and, as Balls has shown (1), air humidity may directly control growth by checking assimilation through the closure of the stomata. Under these conditions available soil moisture no longer constitutes the limiting factor, and it is open to question whether the method would apply. Zaitsev, working on cotton under irrigation in Tashkent, has shown that, under those conditions, temperature is the dominant factor in the growth of the plant (25).

(3) *Soil Conditions.*– A large amount of work has been carried out on the relation between soil moisture and the physical condition of the soil on the one hand, and, on the other, the availability of that moisture for plant growth. Without entering into any detail, it may broadly be stated that light soils, into which water penetrates rapidly, are soils from which water percolates rapidly, and are further soils which support plant growth with a lower percentage of moisture than heavy soils, in which these characteristics are reversed. If, therefore, by writing off a constant percentage of the rainfall, too small an allowance is made for the penetration of water, too small an allowance will, likewise, be made for the losses. The significance of an excess under these conditions immediately following a fall of rain is relatively speaking unimportant for that excess soon passes, while the deficit after a prolonged period of drought corresponds to a condition in which the plant is capable of growing on relatively low values of soil moisture. The two factors, therefore, to some extent neutralise each other.

But this is not the only aspect. It has long been recognised that water is held in the soil in at least two states, which have been termed " free," the physiological water of Schimper, available for plant growth, and the " unfree," not so available. Soils vary markedly in their capacity to retain water in the latter condition, and the major causes for this difference have been traced to the humus content (Crump (6)) and the soil colloids (Keen (12)). Numerous efforts have been made to evolve a simple expression which will define the capacity of a soil to resist the withdrawal of water by the plant ; of such a nature are the " wilting coefficient " and the " moisture equivalent " of Briggs and Shantz (4) and Briggs and McLane (3).

In the work to which reference is here made, the subject is approached from the physical aspect; the present approach is from the biological aspect and a value, k, has been attained which is a measure, not of the "unfree," but of the "free," water of the soil. The utility of the wilting coefficient, as Blackman has shown (2), is subject to definite limitations and, in like manner, the utility of the effective rainfall is circumscribed. As a measure of area the effective rainfall owes its value to the fact that k is not a constant for all soils, while, as a measure of yield, it owes its value to the fact that, over a limited area, a condition which the two analyses of yield satisfy, k has a constant value.

The value given to k, 19/20, is one which satisfies a considerable range of soils in the tropics. It is a value which is readily, though empirically, reached by considering the limiting condition where natural moisture is just sufficient to ensure germination. Accepting the numerical value at this point to be 0·5 inch, it is not difficult to calculate what value of the rainfall must be written off to arrive at the figure 0·5 in the number of days intervening between the previous rain and the date of sowing. The determination of k is, thus, for the present, made on biological grounds.

But plants differ in their water requirements and it follows that, accepting a certain value for k, it will be possible to build up a specific or varietal double curve, giving the upper and lower limits of the water requirements of different species and varieties of agricultural plants at each stage of their growth. One example has been given above for the short-season Indian cotton grown on the Dhulia farm. The American type of cotton, with its longer growing season, possesses a curve of a perceptibly different form, though retaining the bimodal character.

Opportunity has been lacking for a detailed study of this varietal aspect, but already sufficient information has been obtained to permit the employment of the effective rainfall curve for the solution of economic problems. It is fortunate that rainfall records are among the first data to be collected in a new country, and, in consequence, the material is available for working out the curve. From those curves it has been found possible to determine with assurance the best dates for sowing a crop where it has not previously been tried; with further knowledge, it should be possible, with like assurance, to select the variety best suited to any particular moisture conditions.

VII.—*Summary.*

(1) The "effective rainfall" is a daily measure of the soil moisture which originates in rain and is available for plant growth. It is derived from the rainfall data in the following manner

$$E_n = r_n + kr_{n-1} + k^2r_{n-2} \dots + k^{n-1}r_1$$

where r_n is the rainfall of the 24 hours ending 8 a.m. on day n.

(2) The amount of rain written off between successive days is

$$(1 - k)\,r_{n-1} + (1 - k^2)\,r_{n-2} \dots + (1 - k^{n-1})\,r_1, \text{ or } (1 - k)\,E_{n-1}.$$

It includes the loss from run-off, percolation, evaporation and transpiration as well as the moisture held in the soil in the "un-free" condition. k, therefore, is a function of temperature, air humidity and soil conditions.

(3) In part, these sources of loss counterbalance each other, and, for a large range of soils in the tropics, k has a value approximating to 19/20.

(4) Under tropical conditions, k becomes a measure of the "free," or physiological, water remaining in the soil.

(5) For agricultural plants it is possible to draw two curves giving the upper and lower limits of their water requirements at each stage of their growth and the dual curve so obtained has a varietal significance.

(6) As a consequence of that variability of k which arises from soil conditions, it becomes possible to employ the effective rainfall for the estimation of crop areas in a given tract, and an analysis has been made in the case of the wheat and cotton areas of the Cawnpore District, India.

(7) Under conditions which permit the adoption of a constant value for k, the effective rainfall becomes a means of estimating crop yields, and an analysis has been made, in the case of the cane crop, of an estate in Barbados and, in the case of the cotton crop, of the Dhulia Farm, Bombay Presidency.

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CROONIAN LECTURE.—*Certain Problems in the Physiology of the Cerebral Hemispheres.*

By I. P. PAVLOV, For. Mem. R.S.

(Translated into English by G. V. Anrep, F.R.S.—Lecture delivered May 10, 1928.)

It is a great pleasure for me to take this opportunity to offer my hearty thanks to the Fellows of the Royal Society for the help which they gave me during the difficult years through which my country has passed. I wish to thank the Society also for the grant which enabled my last scientific work to be published in English, and for inviting me to deliver the Croonian Lecture.

I believe that Physiology has at last reached a stage at which it is possible to give a general outline of the activity of the entire central nervous system, including that of the cortex of the hemispheres, though as yet, of course, without deep analysis or detailed knowledge of this activity. The primary function of the nervous system is obvious. It is continuously to maintain a dynamic equilibrium between the functional units within the self-contained system of the organism and between the organism as a whole and its environment. The pre-eminent function of the lower parts of the central nervous system is to integrate the activities of the separate parts within the organism. The rôle they play in maintaining the higher animal in equilibrium with its environment is only subsidiary, the most delicate adjustments of this equilibrium being pre-eminently the function of the hemispheres.

A clear and definite proof of this is provided by the old and repeated observation on dogs, in which the cerebral cortex has been extirpated. Such dogs remain in flourishing health, and can probably live as long as normal animals, so high is the co-ordination between the various internal activities of the organism. This, however, can happen only if the animal is under the constant care of man, who must bring its food to its mouth and shelter it from all sorts of harm; otherwise it must inevitably perish. Its powers of adaptation to the environment are very limited. The parts of the nervous system which still remain are insufficient to break up the environment into its elementary units, and to make correlation with its perpetual changes, by establishing temporary connections with the various activities of the organism—for instance, with those of the skeleto-muscular system. The activities of this latter system itself, which is the one chiefly concerned in confronting the environment, now fail to be analysed and synthesised to the same degree as takes place in the presence of the hemispheres. As a result, the dog without hemispheres loses the capacity for fine and precise correlation of each separate act with the separate events occurring outside it.

As a result of these observations it is truly legitimate to distinguish a lower from a higher nervous activity, relating the latter to the hemispheres. An unlimited field opens before the physiologist for investigating the analysing and synthesising aspects of this higher nervous activity of the higher animals, and the mechanism underlying them. This fact of nervous analysis and synthesis has confronted the inquisitive mind of man for a long time. Nervous analysis was the subject of the physiology of the sense organs or receptors of the nervous system, which obviously by their nature also serve the organism as analysers of the environment. The synthesising activity was first formulated by psychologists in the form of the law of association. Thus analysis and synthesis first attracted attention as subjective phenomena. Since then, with the co-operation of many biologists, a method was evolved of strictly objective investigation of these phenomena --a method which can be successfully applied to animals.

The fundamental nervous phenomenon, the use of which renders such an investigation possible, is what I call the *conditioned reflex.* The phenomenon itself was known long before It is an act of synthesis by the hemispheres of the animal. Given that there is a coincidence in time of any external stimulus whatever with some definite activity of the organism, this activity tends to become evoked by that stimulus. I, in co-operation with a great number of co-workers—to whom I send from here warm and sincere greetings—founded on this fact a systematic investigation of the functioning of the hemispheres under both normal and pathological conditions.

We have concerned ourselves mainly with two activities of the organism, namely, its reaction to food and its reaction to substances which are rejected on introduction into the dog's mouth—that is, with the alimentary and with one of the defence reactions--and we connected with these all sorts of stimuli that occurred to us. Food, as a stimulus which acts in its own right from birth, evokes a definite reaction of the animal. The animal takes it into its mouth, masticates and swallows it, and at the same time a secretion of saliva occurs. This reaction we call an unconditioned reflex. If, during the act of eating, some sight or sound or touch affects the animal on each of several occasions, we find that these stimuli become signals of food evoking the same movements and the same salivary secretion. In our experiments we measured only the secretory reaction.

During the last twenty-seven years we have collected an immense number of observations, which it would be impossible to describe even in the shortest form; nor is it necessary, since this would merely be a repetition of what has been said in my recent book. I shall therefore restrict myself to those problems in the physiology of the hemispheres concerning which we have obtained new facts since the appearance of the book.

As the foundation of the activities of the hemispheres we recognise the processes of excitation and inhibition, their movement in the form of irradiation and concentration, and their mutual induction. At present we are obliged to refer special cases of the activity of the hemispheres to one or other of these heads, but no doubt this classification will have to be modified and probably simplified.

Before discussing the actual problems of the present Lecture, I wish to emphasise one important point. More and more observations are being accumulated which show that the establishment of new nervous connections takes place entirely in the hemispheres. The implication is that not only neutral stimuli—*i.e.*, stimuli which are not connected with any activity of the organism—but unconditioned stimuli also, come into communication with definite points of the cortex, pertaining to the respective stimuli. I cannot discuss the evidence for this statement now, but must proceed to the problems which are our immediate concern.

I.

We now know quite well all the conditions under which the conditioned reflex is necessarily established. It follows, therefore, that its establishment is governed by physiological laws, as definite as those which regulate other phenomena in the nervous system. A full and stable conditioned reflex develops when the stimulus which is to become conditioned slightly precedes that activity (unconditioned reflex) with which it is to be linked. The stimulus may also, without ill-effect, terminate a short time before the activity begins (conditioned trace reflex); but if the stimulus is introduced *after* the beginning of the activity, then, although, as our present experiments seem to show, a conditioned reflex may also develop, it is insignificant and evanescent; on continuing the procedure the stimulus, which in this connection we term the neutral agent, becomes inhibitory. This fact, which is at present under careful investigation, *is sometimes strikingly manifested.* If during an experiment we simply repeat short feedings of the animal, not combining them with any external stimulus, no influence is produced either on the general condition of the animal or on the previously established conditioned reflexes. This is shown by tests carried out during the intervals between the feedings. If, on the other hand, some extraneous stimulus is introduced during the actual time of eating, and this is repeated many times, then, after a period varying in different animals, a general inhibition develops: conditioned reflexes weaken conspicuously, and finally disappear completely, the dog even declining food—in fact, there supervenes a hypnotic state. The extraneous stimulus itself, when tested outside the time of feeding, in combination with a positive conditioned stimulus, is found to have become strongly inhibitory. This inhibition can be observed whether the positive stimulus is applied concurrently with the extraneous stimulus or within the period of its after-effect.

Where, under the ordinary method of establishing conditioned reflexes, the conditioned stimulus preceding the neutral stimulus is continued together with it, this, as has been observed from the very beginning of our experiments, never weakens the reflex: on the contrary, this frequently strengthens it.

How are these facts to be understood? From a biological point of view of machine-like reactions of the organism, the interpretation of all these relations does not seem difficult. Since conditioned reflexes play the rôle of signals, they must obviously acquire significance only when they precede in time the physiological activity of which they become signals; and since they act on the extraordinarily responsive cells of the cortex, it would be natural to expect that these cells would not be stimulated longer than necessary, and their energy thus dissipated, but that they should be left to recuperate for another phase of activity. (This suggestion has already been made in my book.) But how should these facts be explained in terms of the general properties of the cortical tissue? How is it that an overlapping in time, given that the neutral stimulus begins to act first, renders this agent an excitatory stimulus, while a similar overlapping when the unconditioned reflex precedes the neutral agent makes the latter an inhibitory stimulus? The following interpretation may be possible.

Negative induction or external inhibition (more and more observations are available to show that these are identical) consists in this, that a stimulation of the cortex at one point leads to inhibition of the rest of the cortex. This would explain how the cells when they are affected by the neutral stimulus, after some definite activity of the cortex has already been started, undergo inhibition: the neutral stimulus, therefore, cannot under these conditions acquire excitatory properties. The mechanism of the development of the conditioned reflex under ordinary conditions can be pictured as follows: the excited state of the cells of the cortex acted on by the neutral agent (when this begins to act first) resists the inhibitory influence of the unconditioned stimulus, and it is only under these conditions that a fusion of the effect of the stimuli takes place, leading to the establishment of a connection between the two points. In other words, the mechanism is based on the confluent irradiation of excitation arising at the two points. This interpretation of the facts, however, leaves many questions unanswered. Why does not the neutral stimulus, when it acts first, evoke inhibition of the points pertaining to the unconditioned stimulus ? Why does it not produce the same effect as that produced by the unconditioned stimulus when this operates first ?

While it is difficult to answer these questions, one can to some extent understand the position by remembering the relative strength of the stimuli ; an unconditioned stimulus is usually much more powerful and more extensive in its effect than the neutral stimulus There is abundant evidence that the relative strength of stimuli is a factor of the utmost importance in the activity of the cortex. Moreover, as I have already mentioned, even where the unconditioned stimulus precedes the neutral agent, an abortive conditioned reflex may appear. Why then, in this case, do the cells pertaining to the neutral stimulus, which is already on its way to becoming a positive conditioned stimulus, invariably pass into a state of inhibition ? A fact of special interest is that, while a neutral stimulus which is introduced at the time of the operation of an unconditioned reflex sooner or later becomes strongly inhibitory, other points of the cortex, which are not stimulated at that time, do not become centres of a strong and protracted inhibition. At the same time, as I have already said, when an established but weak conditioned stimulus overlaps the unconditioned stimulus, its effect becomes, if anything, stronger. The fact that a neutral agent acquires powerful inhibitory properties when it is introduced during the unconditioned reflex (in our experiments on alimentary reflexes) is quite unintelligible from a general biological point of view. It might be suggested that our mode of administration of stimuli is artificial, and that therefore our observations disclose only a sort of pathological exaggeration of a normal mechanism. As against this, however, is the fact that all the temporal combinations of stimuli, which have just been described, frequently occur under normal conditions of life. A satisfactory solution of the problems presented cannot be given without further experimentation.

II.

The second problem with which I propose to occupy your attention relates to the analysing function of the hemispheres. It is obvious that the analysis is based, in the first instance, on the peripheral endings of the various afferent nerves. These peripheral apparatus are a collection of special transformers, in which different forms of energy are changed into nervous energy. Each single afferent nerve fibre, running from some definite element of the peripheral receptive field, must be regarded as a conductor to the cortex of some definite element of one or other form of energy. In the cortex a special cell must stand in connection with the fibre, the activity of the cell being related to some definite element of one or another definite form of energy. This interpretation of the structure of the cortex rests on definite experimental indications: as a result of investigation of functional disturbances of the cortical cells, such a fragmentation of cortical functions is revealed as we could never dream of obtaining by any operative procedure. In my recently published lectures an observation was mentioned showing that it is possible to derange a point pertaining to a separate conditioned stimulus, namely, the sound of a metronome, leaving points corresponding to other auditory stimuli undamaged. Succeeding experiments have confirmed that it is similarly possible to create a localised disturbance of the cortex, corresponding to a definite point in the tactile analyser, without impairment of the normal functioning of any other points. The mosaic construction of the cortex becomes more and more tangible. The further question, however, immediately arises: How far does this spacial differentiation extend, for instance, in the case of different auditory stimuli? We have started, and are continuing, the following series of experiments.

After having produced impairment localised at the cortical point related to a metronome, we proceeded to produce similar impairment at the point related to a particular tone: the selected tone then also ceased to produce a normal effect. It is interesting that in this case the impairment of function involved, to a certain extent, the rest of the tonic scale, so that the reflexes to other tones, which were not used in the experiments, also lost their normal stability—*i.e.*, they easily underwent inhibition. Reflexes to other auditory stimuli, such as buzzing, hissing or bubbling sounds, remained normal. How can we interpret these results except as indicating a precise localisation of different auditory stimuli in the cellular net of the cortex? The facts which I have mentioned are to some extent analogous to some of the various phenomena observed in aphasia in man.

The localised disturbance of the activities of cortical elements can be achieved in two ways. We employ a definite stimulus, which we have reason to believe is related to a definite cortical element, as both excitatory and inhibitory—that is, we develop a differentiation either of frequency of stimulation or of its intensity, and then bring these opposite reflexes into acute collision by applying one frequency or intensity immediately after the other: in certain nervous systems a pathological state of the corresponding cortical point results. The same thing happens when an attempt is made to transform a long-established excitatory stimulus into an inhibitory one, and vice versa. In both cases, as illustrated by instances in my book, the disturbance is the result of a difficult encounter between the opposite processes. Moreover, by mere repetition of a conditioned stimulus for a prolonged period it is possible to render the cortical point more or less permanently inhibited. For instance, on repeating an auditory conditioned stimulus day after day many times in each experiment, it finally became null and void, a condition which lasted for some time. Other auditory conditioned stimuli, however, which were only used infrequently or were temporarily disused, remained entirely unaffected.

I will now refer to another point bearing on the structure of the cortical end of the analysers, namely, vicariation of functions We extirpate some definite convolution of one hemisphere · a generalised cutaneous conditioned reflex suffers definite impairment, conditioned reflexes from some points of the skin lose their positive effect, and stimulation of these places now produces inhibition of all other conditioned reflexes when these are evoked simultaneously or after a short interval. The stimulation of these places may even lead to profound sleep in an animal, which up to this point never slept during the experiments—*i.e.*, it leads to an irradiation of inhibition, not only over the cortex, but over the lower parts of the nervous system. Within weeks or months after the extirpation the positive effect of stimulation at these places returns, but is transformed with extreme ease into inhibition. A few repetitions of stimuli in the same experiment may lead to complete inhibition. In these cases there is no evidence of the possibility of stable differentiation, according to the localisation of stimuli at any of the affected places. Some differentiation is obtained fairly rapidly, but the positive effect soon becomes weak and then vanishes.

The same results of extirpation are at present under observation in a dog which was operated on nearly three years ago. This case is specially instructive, because the operation was not followed by any sign of immediate or late complications in the form of convulsions. In my published Lecture I advanced the conception that, in the cortex, there are, besides the special areas representing the different analysers, certain elements so to speak in reserve which are dispersed over the whole mass of the cortex. I mentioned also that these dispersed elements do not participate in any of the higher synthesis and analysis, functions peculiar to the special areas. As a result of the experiments just described, we are now able to add that the dispersed elements are not even *capable* of reaching the state of functional perfection with which the special areas are endowed.

III.

The next problem in relation to which we have collected new data is that of fluctuations in the excitation of the cortical cells, their transition into an inhibitory state, and the summation of conditioned stimuli. The positive effect of various conditioned stimuli often undergoes considerable fluctuations in strength, even when the conditions apparently remain constant. As we push our investigation further, the necessity of determining the precise cause of every fluctuation becomes more and more imperative. The following is a typical case of which the significance has only recently been appreciated. For a long time it was impossible to find the cause of the fluctuations of different conditioned reflexes evoked during a particular series of experiments. None of the already recognised causes of fluctuation would explain the case in question. Finally, attention was focussed on one of the stimuli as a source of the prevailing disorder in the strength of the reflexes. We began to notice that this stimulus, on being applied first in an experiment, evoked a conspicuously large response, compared with those to other stimuli. If, however, it was repeated in the experiment a second time, its effect was then conspicuously small. Next we noticed that it was just after the application of this stimulus that the irregular fluctuations appeared in the strength of the other conditioned reflexes, and that, in addition, the animal became excited.

All this inclined us to think that the stimulus was a very strong one for the cortical cells of the particular animal: the verification of this supposition was not difficult. It was sufficient to decrease the intensity of the stimulus in order to make the condition of affairs change abruptly. The positive effect of this stimulus diminished somewhat, but it now became considerably more uniform in strength on repetition. Sometimes, even, it did not change at all during the whole of an experiment. The other reflexes also ceased to fluctuate in strength, and the animal quieted down. In order to collect further evidence some of the other stimuli were in turn somewhat increased in strength, and, as a result, the same fluctuations were observed as had been produced by the original strong stimulus. Experiments showing the effects of increased and decreased strength of stimuli can be repeated several times over in the same animal. Having acquired this information, we often, when beginning work with a new animal, tested the strength of various conditioned stimuli. In every separate experiment we repeated the same stimulus several times. In the usual course of things, after several repetitions of a conditioned stimulus in the same experiment, its effect diminishes to some slight extent towards the end of the experiment. The extent of this diminution and the amplitude of the fluctuations during the experiment definitely indicate those stimuli which are excessively strong, and therefore unsuitable for further experiment (unless, of course, the object of the experiment is to test stimuli of excessive strength). In the case of the repetition of excessively strong stimuli the progressive diminution in their effect is very considerable towards the end of the experiment, and during the experiment the fluctuations are remarkably great.

In different animals the agencies which act as extraordinarily strong stimuli may be widely different from one another, as regards their *physical* strength. Every animal, therefore, has a certain limit to what may be called normal excitability, for there is a definite optimal strength of each stimulus. As soon as the individual limit of normal excitability is reached, the corresponding cortical cells become more and more inhibited, and this state is reflected in other cells which are stimulated by other stimuli, with resultant variations in the strength of the reflexes in one direction or another, on account of irradiation or induction. It is therefore obvious that we have constantly to ensure that our conditioned stimuli should remain within the limits of their optimal strength.

In close conjunction with the question of the limits of normal excitability stands that of the summation of conditioned stimuli, which has interested us for a long time, but has not hitherto lent itself to solution or experimentation As suggested in my lectures, the magnitude of the conditioned reflex is determined, *ceteris paribus*, by the amount of energy transmitted from the stimulus to the cortex. The greater the energy, within certain limits, the greater is the conditioned response If two weak conditioned stimuli are applied together, their summated effect approximates to that of a strong stimulus. At certain strengths of weak conditioned stimuli an exact arithmetical summation of effect can be observed. If a weak stimulus is combined with a strong, their summated effect is nearly always equal to the effect of the strong one alone Finally, the summation of two strong stimuli produces an effect which is usually somewhat smaller, and only very seldom greater, than that of either singly.

In the variation of the experiment already described, when, in the course of one experiment, a single conditioned stimulus was repeated many times, the following results of summation were observed: first we obtained several curves expressing the fluctuations in the strength of conditioned reflexes for a weak, a medium and an excessively strong stimulus. Next we combined the action of the weak and medium stimuli, and repeated this summated stimulus the same number of times as the separate stimuli: the curve so obtained is identical in type with the curve for the strongest stimulus, exhibiting very considerable variations during the experiment, and ending in a profound diminution during the last few applications.

There are other phenomena concerned in summation. In the first place, it has a certain after-effect. In the case of a single application of a summated stimulus, the after-effect involves the subsequent reflexes in the same experiment not only those in response to the component stimuli, but also to all others The after-effect is obvious for several days Most conspicuous is the inhibitory after-effect left by the summation of strong stimuli. The conception of the limit of normal excitability of cortical cells throws much light on the details of the fact of summation, but there still arises the further and very difficult question of the point where summation takes place. The results of summation of weak conditioned stimuli might naturally be regarded as a fusion of the effect of both weak stimuli in that point of the cortex with which, in all these particular experiments, the conditioned stimuli are brought into relation viz., the chemical analyser in the cortex. But on the other hand, the summation of the weak conditioned stimulus with the strong, and of the two strong together, definitely points to the cells pertaining to the conditioned stimuli themselves. We have every right to regard the inter-relation of processes in summation as taking place somewhere within the above-mentioned sets of cells, but what is the share of the chemical analyser, and what the share of the cells pertaining to the conditioned stimuli, must be answered by the investigations on which we are now engaged.

When, in the same experiment, we apply alimentary conditioned reflexes in conjunction with the reflexes to acid, the inter-relations become still more involved, because they are complicated by the interactions of the different regions of the chemical analyser itself. The problems thus arising are also under investigation

IV.

Our final problem concerns the types of nervous system. The experimental material, collected from dogs which we used for our observations, is so large that we have a certain basis for defining at least the main types of nervous system. The difference as regards the development and the character of excitatory and inhibitory conditioned reflexes in our animals may be striking. There is one group of dogs in which the positive conditioned reflexes develop with ease, quickly reaching and persistently remaining at their maximum strength, often in spite of various inhibitory influences, *i.e.*, interference from extraneous reflexes. In the case of these animals an attempt has been made to reduce the effect of strong or weak conditioned stimuli by means of unbroken repetition, a method usually very effective for this purpose, but the reflexes remained very steady. The inhibitory reflexes, on the other hand, develop in these animals with great difficulty, and it seems as if the animals' nervous system opposes a barrier to their establishment. Much time must usually be spent in order to establish them firmly, if, indeed, this is possible at all. Some of these dogs fail to develop fully inhibitory reflexes, such, for instance, as those involved in the establishment of absolute discrimination of stimuli. In others fully inhibitory reflexes can be established, but they should not be repeated during a single experiment, or even once a day, otherwise they again lose completeness, and they are very easily disinhibited by extraneous stimuli. This type of animal may be called the excitable type.

At the other extreme is the type in which the positive reflexes develop under our conditions very slowly, slowly reach their maximum strength, and are extremely liable to diminish and disappear for considerable periods of time, in the presence of quite insignificant extraneous stimuli. Frequent repetitions of the excitatory reflexes also lead to their diminution and disappearance. The inhibitory reflexes, on the other hand, are developed extraordinarily quickly, and well maintain their strength This type of animal may be called inhibitable. As regards the cortical cells of these two groups of animal, it may be presumed that in the excitable type the cells are vigorous and richly provided with "excitable substance," while in the inhibitable type the cells are weak and poor in that substance. For these weak cells the usual strength of stimuli is super-maximal, and hence leads to inhibition.

In between these two extreme types is the central type. This easily acquires both positive and negative conditioned reflexes, which, after development, are stable. Since the normal nervous activity consists in a perpetual equilibration of the two opposing nervous processes, and since in the last type this equilibration is more or less easily achieved, we may call this the "well-balanced" animal. We have at our disposal several criteria for comparing different animals as regards their conditioned activity, and the grouping of animals which I have just indicated finds constant confirmation. Of course, there are several gradations between these primary types. This classification also finds support in the fact that all the characteristic differences become exaggerated under the influence of various prolonged nervous disturbances (experimental neuroses) which develop as the result of excessively strong stimulation, or of unresolvable conflict between the two nervous processes. The balanced type more or less quickly, and at any rate without lasting disturbance, overcomes these difficulties; the extreme types show definite neuro-pathological symptoms differing in the two types. The excitable type entirely loses all capacity for inhibition, and enters a state of strong and continuous excitation, both under the conditions of our laboratory environment and at large; the inhibitable type, on the contrary, loses almost completely the positive conditioned reflexes, and, in response to conditioned stimuli, passes through various phases of the hypnotic state. Treatment is needed in order to restore these animals to the normal—prolonged rest and interruption of the experiment, or pharmaceutical remedies, or both.

It is of interest that the balanced type, as judged by means of our tests, is represented in two groups of animal, varying greatly in their general behaviour—one stolid and quiet, peculiarly indifferent to external happenings, but always on the alert; the other extraordinarily lively and mobile under ordinary conditions, and showing continual interest in whatever happens around them, but under monotonous conditions—for instance, when left alone in the experimental room—surprisingly apt to fall quickly asleep. These dogs, like the quiet ones, though not so easily, overcome the difficulties presented to them.

It is obvious that these types of nervous system are what is usually defined as "temperaments." Temperament is the most general characteristic of an individual, whether man or animal. It is the most fundamental characteristic of the nervous system, a characteristic which colours and pervades all the activities of every individual. This being so, we cannot fail to see that our types correspond to the ancient classification of temperaments, the choleric and melancholic types being our extremes—excitable and inhibitable; while the phlegmatic and sanguine correspond well to the two forms of balanced type—the quiet and the lively. It seems to me that our classification of temperaments, which is based on the most general properties of the central nervous system, namely, the relations between the two aspects of nervous activity, inhibition and excitation, is the most simple and the most fundamental possible.

Now since, in our experiments with conditioned reflexes, we are concerned with the properties of the hemispheres, we can go a step further and say that temperament is determined mainly by the properties of these. That the facts of temperament are not attributable to special complex peculiarities of the unconditioned reflexes, usually known as instincts or tendencies, is shown by the fact that the unconditioned alimentary reflex may be very intense in extremely inhibitable animals. Complex and special manifestations of unconditioned reflexes, such as the alimentary, the defensive both in its active and passive form, and others, depend of course on the activity of the higher sub-cortical centres, which serve as the basis of the elementary emotions. The sum total of the vital expressions, however, will be mainly dependent on the type of the activity of the cortex, which may be predominantly excitatory or inhibitory, or both in different proportions, and which modifies the sub-cortical activity accordingly.

The conception of the preponderant significance of the fundamental properties of the cortex, as determining temperament, should be accepted as applicable to man.

Having completed this survey of our recent experiments, and of the series of new problems arising therefrom in the physiology of the hemispheres and of the brain generally, I will attempt to draw two general conclusions—one purely physiological, the second more practical and of a certain general application.

If the central nervous system is to be divided into two parts only, the afferent and the efferent, then I would regard the cortex of the hemispheres as constituting an isolated afferent area. In this area only does the higher analysis and synthesis of the inflowing excitations take place, and it is only from here that ready-made combinations of excitation and inhibition can flow into the efferent areas. In other words, only the afferent part is the active or, so to speak, the creative part, while the efferent is the obedient executive. In the spinal cord the afferent and efferent parts are intimately connected: the investigator always carries away the impression of a unified activity of both parts, and is, as I believe, precluded from giving a self-contained description of the peculiarities of the afferent part. For instance, the law of forward conduction of the nervous process is substantiated by experiments demonstrating the uninterrupted progression of the spinal reflex act from start to finish. But is this law valid for a purely afferent organ? In the cortex of the hemispheres we continually observe both progression and regression of the excitatory and inhibitory processes. Is this the result of two-directional conduction along the same paths or, in order to preserve the principle of the law of forward conduction, must we understand that special complicated constructional devices come into play ?

Coming to the more practical of the two conclusions which I have mentioned, I am led to it under the influence of persistent impressions, formed over a long series of years devoted to this work. These multitudinous experiments on the activity of the hemispheres reveal the astounding plasticity of this activity. Many problems depending on the nervous function, which may seem for a given brain entirely impossible of solution, nevertheless, by means of gradual presentation and careful method, become in the end satisfactorily solved : and if they are to be solved, the type of nervous system of the individual animal must never be ignored.

I trust that I shall not be thought rash if I express a belief that experiments on the higher nervous activities of animals will yield not a few directional indications for education and self-education in man. I, at any rate, can say, looking back on these experiments, that for myself they have made clear many things, both in myself and in others.

The Chemical Composition of Vegetable Seed Fats in Relation to the Natural Orders of Plants.

By Thomas Percy Hilditch, D.Sc.

(Communicated by Dr. E. F. Armstrong, F.R.S.—Received May 1, 1928.)

(From the Department of Industrial Chemistry, University of Liverpool.)

Analyses, by recent and improved methods, of the mixed fatty acids from various seed fats are now available in a number of different cases, and show, more definitely than hitherto, that the seed fats of members of the same botanical group frequently possess strongly marked specific resemblances.

It has been recognised, of course, for a considerable time that seed fats from plants belonging to the same or nearly allied botanical orders often contain similar, and to a certain extent specific, mixtures of fatty acids. Thus, the fats from fruits of the *Palmæ* are marked by the presence of relatively large quantities of lauric acid, whilst myristic acid is prominent in those of the *Myristiceæ*, and erucic acid in seeds of the *Cruciferæ*.

Recent studies by investigators working in collaboration with the present writer, together with similar data due to other workers, indicate that, so far as the *Palmæ* and *Cruciferæ* are concerned, the relative proportions of all the fatty acids present are strikingly similar, whilst in seeds of the *Umbelliferæ* the occurrence, in varying amounts, of a peculiar isomeric form of ordinary oleic acid ($\Delta^{6:7}$-octadecenoic acid or petroselinic acid) has been established in every instance so far examined. The present communication offers a conspectus of the data at present available in regard to the four botanical orders mentioned.

Seed Fats of the Palmæ.

This is the most familiar case of family resemblance in the chemical structure of seed fats, because of the large extent to which the so-called nut oils from kernels of genera in this order are used in industry, in virtue of their soft but solid texture at the ordinary temperature. These features are due, of course, to the presence of a large percentage of glycerides of fatty acids of lower molecular weight than that of oleic acid and in particular to large amounts of combined lauric acid. Such resemblances only pertain to the kernel fats (*cf.* Armstrong and Allan*) : the fat from the pericarp of the fruit may have quite different characteristics, including the entire absence of lauric and lower acids Further, the fat of the testa is usually different from that within the kernel (Richardson,[*] Armstrong and Allan,[†] Allan and Moore[‡])

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* Armstrong and Allan, 'J.S.C.I.,' vol. 43, p. 211T (1924).

The characteristic low molecular weight fatty acids of kernels of the *Palmæ* are, in fact, concentrated within the endosperm of the fruit, and it further seems not improbable that this true endosperm fat has a very closely similar composition in all cases, and that the amount of oleic acid present therein is not more than about 5–10 per cent of the total combined fatty acids This is well illustrated by the general characteristics (saponification equivalent and iodine value) of the fats from the testa and testa free kernels of the following nuts (as recorded by Allan and Moore[§]) –

Kernels	Saponification equivalent		Iodine value	
	Testa fat	Testa free fat	Testa fat	Testa free fat
Palm kernels	244 0	229 0	28 0	12 3
Babassu kernels	241 0	217 5	22 8	10 2
Curucury kernels	232 5	214 0	30 4	10 5

The detailed compositions of the combined fatty acids present (for which data are now available for palm kernel fat from kernels with testa, for coconut fat both from the testa and from the testa free kernels and for cohune nut fat from kernels with testa) renders the comparison still more striking —

—	Coconut *		Palm kernel* (with testa)	Cohune† (with testa)
	Testa fat	Testa free		
	Per cent	Per cent	Per cent	Per cent
Caprylic acid	2 (?)	9 5	3 0	7 5
Capric acid	2	4 5	3 0	6 5
Lauric acid	28	51 0	52 5	46 5
Myristic acid	22	18 5	15 0	16 0
Palmitic acid	12	7 5	7 5	9 5
Stearic acid	1 (?)	3 0 (?)	2 5	3 0
Oleic acid	23	5 0	16 0	10 0
Linoleic acid	10	1 0	1 0	1 0

* Armstrong, Allan and Moore, J S C I, vol 44, pp 61T, 143T (1925)
† Hilditch and Vidyarthi, 'J S C I,' vol 47, p 35T (1928)

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[*] Richardson, J Ind Eng Chem,' vol 3, p 574 (1911)

[†] *Loc cit*

[‡] Allan and Moore, 'J S C I,' vol 44, p 61T (1925)

[§] *Loc cit*

The main component of each kernel fat is lauric acid, $C_{12}H_{24}O_2$ (46·5 to 52 per cent. of the mixed fatty acids), the next most prominent acid is myristic, $C_{14}H_{28}O_2$ (15–18·5 per cent.), followed by palmitic acid $C_{16}H_{32}O_2$ (7·5–9·5 per cent.), and oleic acid, $C_{18}H_{34}O_2$ (from 5 per cent. upwards). There are smaller quantities of caprylic acid, $C_8H_{16}O_2$, and capric acid, $C_{10}H_{20}O_2$, amounting together to from 6–14 per cent. of the total fatty acids.

The characteristics of other tropical nut fats examined by the Imperial Institute* suggest that widely differing genera of the *Palmæ*, grown in districts so widely separated as the East Indies, West Africa, and Central or South America, contain fatty acid mixtures of the same nature :—

Name.	Source.	Habitat.	Kernel fat.	
			Sap. equiv	I.V.
Cokerite nuts	*Maximiliana* sp.	British Guiana	222	13·0
Babassu kernels	*Attalea funifera*	Brazil	225	15·6
Tucan nuts	*Astrocaryum vulgare*	Brazil	225	11·6
Paraguay kernels	*Acrocomia* sp.	Paraguay	227	28·5
Noli Palm	*Elæis melanococca*	Colombia	240	27·7
Curua palm	*Attalea spectabilis*	Brazil	216	8·9
Guere palm	*Astrocaryum* sp.	Colombia	225	9·4
Mamarron	*Attalea* sp.	S. America	224	10·8

This close connection between botanical and chemical characteristics—the more remarkable since it involves a specific type of fatty acid mixture (high lauric acid content) not met with in any other natural fat—is probably reinforced by general similarity in the component glycerides present in the fats. Knowledge of the mode of union of fatty acid with glycerol in natural fats has hitherto been scanty, but work in progress in this laboratory† on palm kernel and coconut fats goes to show that in both cases by far the larger part of the lauric and myristic (and most of the caprylic and capric) acids is combined in the form of saturated triglycerides, *i.e.*, independently of oleic acid ; the latter acid is linked with about its own weight (about 1·3 equivalents) of saturated acids (chiefly lauric and palmitic) ; simple triglycerides (*e.g.*, trilaurin or triolein) appear to be absent.

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* 'Imperial Institute Bulletins,' vol 14, p. 8 (1916) ; vol. 15, p. 38 (1917) ; vol. 17, p. 186 (1919) ; vol. 18, p. 172 (1920) ; vol. 19, p. 293 (1921) ; vol. 20, p. 147 (1922).

† Collin and Hilditch, forthcoming publication.

Seed Fats of the Myristiceæ.

No data based on modern methods have yet been given for the detailed composition of the combined fatty acids of fats of the mace family, such as nutmeg butter; but records of their general characteristics (such as the saponification equivalent and iodine value) indicate the presence of large amounts of combined myristic and oleic acids, as shown in the next table :—

Seed fat from	Common name.	S.E.	I.V.	Habitat.
Myristica officinalis[*]	Common nutmeg	248	7·0	E. Indies.
„ *argolensis*[†]	Kombo fat	262	77·4	Nigeria.
„ *guatemalensis*[‡]	Virola fat	253	12·4	Venezuela.
„ *bicuhyba*[§]	Ucuhuba fat	255	14–18	Brazil.
„ *otoba*[‖]	Otoba fat	243	?	America.
Scyphocephalium ochocoa[¶]	Ochocho fat	235	1·7	W. Africa.

[*] Fabris and Settimj, ' Atti del VI Congresso internaz. di chimica applicata,' Rome, p. 756 (1907).
[†] Imperial Institute, ' Bulletin,' vol. 6, p. 378 (1908).
[‡] Grimme, ' Chem. Revue,' vol. 17, p. 233 (1910).
[§] Lewkowitsch and Warburton, ' Oils, Fats & Waxes, 6th edit., vol. 2, p. 580 (1922).
[‖] Baughman, Jamieson and Brauns, ' J. Amer. Chem. Soc.,' vol. 43, p. 199 (1921).
[¶] Lewkowitsch and Warburton, *ibid.*, p. 581.

The *Myristica* seed fats therefore appear to stand apart in their high contents of combined myristic acid (an acid which does not occur in large proportions in any other type of vegetable fat); and considerable proportions of the myristic acid are stated to be united with glycerine separately from oleic acid (Power and Salway,[*] Verkade and Coops[†]). In this way the seed fats of this family appear to run more or less parallel with those of the *Palmæ*, except that the characteristic lauric acid of the latter is here replaced by myristic acid.

It may be added that a re-investigation of some of the mace fats by the aid of recent methods is contemplated at an early opportunity by the author and co-workers.

Seed Fats of the Cruciferæ.

The fatty oils of the *Cruciferæ*, widely remote in general properties and chemical structure from the tropical nut fats, are also characterised by resemblances peculiar to the family, notably their high content (usually 40–50 per cent.) of combined erucic acid, $CH_3[CH_2]_7CH : CH . [CH_2]_{11}CO_2H$, and also the occurrence of very small amounts of an acid (or mixture of acids) isomeric, but not identical, with ordinary oleic acid.[‡]

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[*] Power and Salway, ' J. Chem. Soc.,' vol. 93, p. 1653 (1908).
[†] Verkade and Coops, ' Rec. trav. chim.,' vol. 46, p. 528 (1927).
[‡] Hilditch, Riley and Vidyarthi, ' J.S.C.I.,' vol. 46, p. 462T (1927).

Some detailed analyses (which have become available only recently, although the presence of erucic acid in *Brassica* seed oils has been recognised for many years) are appended. These illustrate the striking similarities, coupled with subordinate differences, in the composition of fatty acids from rape seed, black and white mustard, and common wallflower seed oils.

Botanical species.	*Brassica campestris.*			*B. nigra.*		*B. alba.*	*Cheiranthus cheiri.*
Description.	Rape.		Ravison	Black mustard.		White mustard.	Wallflower.
	English.*	Indian.†	Danubian.†	English.*	Indian.†	English.†	English.‡
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Fatty acids.							
(i) *Saturated*—							
Myristic	—	1·5	—	—	0·5	—	—
Palmitic	1	--	2	2	—	2	3
Stearic	—	1·6	—	Trace	—	Trace	—
Arachidic	—	—	—	Trace	—	1	—
Behenic	—	0·5	Trace	--	3·8	—	—
Lignoceric	1	2·4	2	2	1·1	1	0·5
(ii) *Unsaturated*—							
Oleic	32	20·2	20·5	24·5	32·3	28	12·5
Linoleic	15	14·5	25·5	19·5	18·1	14·5	41
Linolenic	1	2·1	2	2	2·7	1	4
Erucic	50	57·2	47	50	41·5	52·5	39

* Hilditch, Riley and Vidyarthi, 'J S.C.I.,' vol. 46, p. 457T (1927).
† Sudborough, Watson and Ayyar, 'J. Ind. Inst. Sci.,' vol. 9A, p. 25 (1926).
‡ Hilditch and Jones, 'J.S.C.I.,' vol 46, p. 467T (1927).

Although it is not safe to formulate precise conclusions on the basis of the mean characteristics of a fat as a whole, it appears highly probable from the saponification equivalent, iodine value, and content of non-saponifiable matter present in seed fats of seven other members of the *Cruciferæ* which were examined by Grimme,* that other fatty oils of the *Cruciferæ* share in the peculiarities of those which have been more intensively examined. At all events, the seed fats of all Cruciferous plants which have been closely examined up to the present bear very close resemblances to each other, and at the same time are distinct in chemical composition from those of any other botanical order; except that, according to Sudborough, Watson, Ayyar and Damle,† the seed fat of *Tropæolum majus* has a composition by no means dissimilar from those of the *Brassica* fats.

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* Grimme, 'Chem. Rev. Fett. Harz. Ind.,' vol. 19, p. 102 (1912).

† Sudborough, Watson, Ayyar and Damle, 'J. Ind. Inst. Sci.,' vol. 9A, p. 65 (1926).

Seed Fats of the Umbelliferæ.

So far as is known at present, the fatty acid mixtures comprised in these seed fats are comparatively simple, in that they consist almost exclusively of unsaturated acids of the C_{18} series; but a peculiar feature is that an important proportion of the oleic acid (*e.g.*, from 20–80 per cent.) is not the ordinary $\Delta^{9:10}$-octadecenoic acid, but an isomeric $\Delta^{6:7}$-octadecenoic acid.

Vongerichten and Kohler* first isolated this acid ("petroselinic acid") from the fatty oil of parsley seeds (*Petroselinum sativum*); petroselinic acid forms about 75 per cent. of the total fatty acids of parsley seed oil,† and its structure has been confirmed by Miss E. E. Jones and the writer,† and by van Loon.‡

The seeds of *Pimpinella anisum* and *Fœniculum capillaceum* were stated by Scherer§ to contain a solid oleic acid (apparently identical with petroselinic acid); the latter acid was also found by Palazzo and Tamburello|| in seeds of the common ivy (*Hedera helix*), belonging to the *Araliaceæ*, an umbellate family closely allied to *Umbelliferæ*, although separated on account of the more succulent fruit.

Other Umbelliferous seed fats are being studied in this laboratory. Those of the native wild species *Heracleum sphondylium* and *Angelica sylvestris* each contain about 20 per cent. of $\Delta^{6:7}$-octadecenoic acid,¶ whilst those of cultivated species, such as carrot, celery, chervil and parsnip, also contain varying amounts (30–50 per cent.) of this acid.**

Clearly this is another case in which definitely related groups elaborate a more or less specific type of fatty acid for purposes of storage of the fatty matter in the fruit; but in *Umbelliferæ* the specific type takes the form, not of a particular acid, lower or higher in the homologous series of fatty acids than the customary C_{16} or C_{18} acids, but of a definite oleic acid isomeric with the more generally distributed $\Delta^{9\cdot10}$-octadecenoic acid, existing concurrently with the latter, and so far encountered only in the seed fats of this particular order, *Umbelliferæ*, and of the very nearly related *Araliaceæ*.

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* Vongerichten and Kohler, 'Ber.,' vol. 42, p. 1638 (1909).

† Hilditch and Jones, 'J.S.C.I.,' vol 46, p. 174T (1927).

‡ Van Loon, 'Rev. Trav. Chim.,' vol. 46, p. 492 (1927).

§ Scherer, 'Inaugural Dissertation,' Strassburg (1909).

|| Palazzo and Tamburello, 'Atti R. Accad. Lincei,' vol. (v), 23 (ii), p. 352 (1914).

¶ Hilditch and Jones, 'Biochem. Journ.,' vol. 22, p. 326 (1928).

** Christian and Hilditch, forthcoming publication.

Conclusion.

The evidence at hand is still somewhat scanty and diffuse, and from the nature of the case it is difficult to accumulate detailed analyses of the combined fatty acids in various seed fats at anything but a very slow rate. Nevertheless, the facts set out above render it evident that, in the four orders discussed, there is a marked tendency towards the production of quite specific fatty acids. Other acids, of course, are also present, oleic and linoleic acids usually in fair to considerable proportions, and also minor amounts of such acids as palmitic, arachidic or lignoceric; but the four acids, lauric, myristic, erucic and petroselinic, stand out quite definitely in their nature and proportion in the respective cases of the four orders, *Palmæ*, *Myristiceæ*, *Cruciferæ* and *Umbelliferæ*.

It is to be inferred that the seed fats of any given botanical order have certain characteristics of their own, and much in common with each other, differing from those of dissimilar orders.

Myothermic Apparatus.

By A. V. Hill, F.R.S.

(Received May 8, 1928.)

(From the Department of Physiology and Biochemistry, University College, London.)

The investigations to be described in subsequent papers represent an attempt to clear up, with the greatest accuracy possible, a number of outstanding or controversial points in connection with the energy exchanges of muscle. During the course of them a new and striking phenomenon has been encountered, in respect of the resting heat-production of muscles kept under strictly anaerobic conditions. It has been necessary, moreover, for various purposes, to follow the heat-production of stimulated or recovering muscles for long periods, sometimes for an hour or more. The apparatus available proved inadequate for these new purposes, and had to be designed and constructed afresh.

The present paper is a description of the methods finally adopted; the results obtained are given separately. In almost every respect the apparatus now employed will yield more reliable results, and is simpler to use, than any previously described, at any rate by the present author. The essential condition which it fulfils is that it will read, with relative accuracy, not only the heat suddenly produced by a single stimulus, but that liberated over long intervals at rest, or in recovery, or by prolonged discontinuous stimulation.

A.—*Galvanometer*.

Throughout the present experiments, which have required considerable zero-stability in the recording instruments, the galvanometer employed has been of the moving-coil type, viz., a Zernicke (Zd) galvanometer manufactured by Messrs. Kipp of Delft. With a complete period of about 2¼ seconds, with a resistance of about 25 ohms, and with its magnetic shunt adjusted for critical damping with an external resistance of about 60 ohms, this instrument gives, at 3 metres distance, a deflection of 40 to 80 mm. for a single muscle twitch, when connected to a thermopile of the type described below. The galvanometer was mounted on a Julius suspension, damped by a vane in oil beneath, care being taken to place the point of attachment of the coil to the body of the galvanometer as near as possible to the centre of gravity of the whole suspended system. This arrangement abolished mechanical disturbances to such a degree that reading was possible to 0·5 mm. at a distance of 3 metres. Nothing but heavy gusts of wind produced a measurable disturbance, in spite of the fact that all the experiments were performed on the third floor of the building, with no other precautions taken to avoid mechanical vibrations.

The coil of this galvanometer—as is necessary for its short period—is of extreme lightness, and perhaps for this reason, in spite of a quartz fibre suspension, its zero shows a gradual creep (up to 5 mm. in a period of hours), especially when the light is first turned on. This is probably due to slight alterations in the mechanical state of the coil, caused by changes of temperature. To avoid error the scale was adjusted to galvanometer-zero at intervals throughout an experiment.

The mirror of the galvanometer is sufficiently large and plane to allow the beam reflected from a "pointolite" lamp to be read to 0·2 mm. in broad daylight on a scale at 3 metres. In certain cases, as, for example, in measuring the total heat produced in a series of twitches at 3-second intervals, it is desirable to employ an overdamped deflection of the galvanometer, in order to smooth the curve obtained by reading every 5 seconds. This is readily done by increasing the magnetic field, and, if necessary, introducing an appropriate series resistance and shunt.

Several galvanometers of this type, possessing various characteristics, are manufactured by Messrs. Kipp. They seem, for myothermic measurements, to be greatly superior to any other moving-coil type; they possess a short period, good zero-keeping qualities and absence of creep, a high sensitivity, a good large mirror, and a magnetic shunt for adjusting sensitivity and damping, and they are simple to use and control. Being—like all moving-coil galvanometers—completely unaffected by external magnetic fields, they eliminate one serious source of error. Where great quickness of response, or extreme sensitivity, is required, they are still considerably inferior to a good moving-magnet galvanometer, *e.g.*, of the type designed by Downing (1). For general purposes, however, in myothermic measurements, their various advantages, and their simplicity in the hands of a novice, make them far the best recording instrument at present available. Further details of their performance have been given by Gerard (10), who used a pair of them, with a thermal relay, for the analysis of the two phases of nerve heat-production.

B.—*Recording.*

The initial heat produced in a single contraction of a muscle (twitch or tetanus) was read directly as a deflection on a scale, or else recorded photographically in case an analysis of the time-relations of the heat-production was required. For photographic recording a camera by Boulitte was used, employing rapid bromide paper 8·9 cm. wide wound on a drum 100 cm. in circumference. One advantage of this camera is that it can be used in daylight, with a shutter to cut out the light when the record is not actually being made. It was driven at the desired speed by a motor, and the beam of light from a narrow vertical slit in front of a " pointolite " lamp was recorded as a sharp black line, interrupted by a metronome ticking half-seconds. The camera was distant about 70 cm. from the galvanometer. In many experiments it was desired to record the early part of the deflection (15 to 30 seconds) photographically, and to read the rest of it (up to 6 minutes in nitrogen or 25 minutes in oxygen) at suitable intervals on a scale. This was done by employing *two* reflected beams of light, one falling on the camera, the other on the scale. The maximum read on the scale was compared with that of the photograph, and so the two records were fitted together.

The light was admitted to the camera by the magnetic release of a shutter, timed to coincide with the beginning of the stimulus. By spacing the records suitably five could be made on a single strip of paper. The simultaneous reading on the scale gave a considerably improved control of the experiment, the observer being able to assure himself that everything went according to plan, without moving from his usual position. A base line was provided on the photographic record by making an exposure with the galvanometer disconnected from the thermopile. The records were perfectly smooth and sharp, and readable easily to 0·1 mm., the time-marks were quite clean-cut, and the commencement of the stimulus clearly shown by the sudden beginning of the curve.

In many of the experiments to be described the *total* heat was required, *e.g.*:

(*a*) in a series of twitches in oxygen or nitrogen;

(*b*) in a single contraction, for comparison with the initial heat;

(*c*) in oxidative recovery after anaerobic stimulation; and

(*d*) when carbon dioxide was admitted, and combined with the alkalies of the muscle.

The *total* heat is given by the area of the deflection-time curve, over any complete process, as the following considerations show.

The flow of heat, the rise and subsequent fall of temperature of the hot junctions, and the movements of the galvanometer in response to the sudden production of an element of heat δH, are all governed by equations which, if we could solve them, would lead to an expression of the following type for the galvanometer deflection δy as a function of the time t:—

$$\delta y = \delta H f(t).$$

If a number of heat elements $\delta H_1, \delta H_2, \delta H_3, \ldots$, be liberated at times $\theta_1, \theta_2, \theta_3, \ldots$, the resulting total deflection is given by

$$\begin{aligned} y &= \delta y_1 + \delta y_2 + \delta y_3 + \ldots \\ &= \delta H_1 f(t - \theta_1) + \delta H_2 f(t - \theta_2) + \delta H_3 f(t - \theta_3) + \ldots \end{aligned}$$

Now the total area Y of the deflection-time curve is $\int_{t=0}^{t=\infty} y\,dt$, and is, therefore,

$$Y = \delta H_1 \int_{t=0}^{t=\infty} f(t - \theta_1)\,dt + \delta H_2 \int_{t=0}^{t=\infty} f(t - \theta_2)\,dt + \ldots.$$

But the integrals are all equal, provided (i) that $f(t - \theta)$ is zero for $t < \theta$, as is obviously the case; and (ii) that the times $\theta_1, \theta_2, \theta_3, \ldots$, do not continue to ∞, *i.e.*, that the total heat is liberated in a finite time. Hence we have finally,

$$Y = (\delta H_1 + \delta H_2 + \delta H_3 + \ldots) \int_{t=0}^{t=\infty} f(t)\,dt,$$

or in other words, the total area Y of the deflection-time curve is proportional to the total heat set free, whatever its distribution in time. It should be particularly noted that this relation refers only to *total* areas ($t = 0$ to $t = \infty$), and that the area of any *part* of the deflection-time curve is *not* necessarily a measure of the heat liberated in the corresponding interval. To obtain the latter, a full analysis of the heat-production is necessary, which is possible only by the laborious numerical method described by Hartree and Hill (2) (3).

The area of the deflection-time curve was obtained by plotting on squared paper the deflection read at suitable intervals on the scale. It is necessary that the deflection should be constant at the beginning, and at the end, of the series of readings. The areas were evaluated either (i) with a planimeter, (ii) by cutting out and weighing, or (iii) by direct addition. It is usually necessary to allow for the resting heat-production, which may not remain constant: the manner of doing this varies from one type of experiment to another, and will be described in the proper place.

As a corollary to the above it is obvious that when the rate of heat-production is constant there will occur a constant deflection on the scale. Thus the *rate* of resting metabolism—if constant—can be read directly at any moment, as a displacement from galvanometer-zero.

The theoretical relations described above have been verified experimentally, and found to be strictly true.

C.—*Thermostat.*

In order to obtain a constant zero, from which to read the resting heat-rate, it is essential that no differences of temperature should persist within the thermopile. The absence, moreover, of temperature fluctuations greatly increases the accuracy of the determination of the recovery heat-rate over long intervals. To avoid such temperature differences, the thermopile, as described below, may be constructed mainly of good heat-conductors. There is, however, a limit to the improvement obtainable by such means: heat conduction from hot to cold junctions in the thermopile *must* be relatively slow, otherwise heat produced by the tissue on the hot junctions is too rapidly lost, and the sensitivity is proportionally diminished. Even the best thermopile hitherto constructed, placed in a Dewar flask, under water well stirred but otherwise uncontrolled in temperature, exhibits temperature differences far too great to allow an accurate determination of the resting heat-rate. The gradual change in temperature of the bath, and therefore of the thermopile, causes a heat flow along the latter, with persistent temperature differences between hot and cold junctions. To avoid such errors Hartree has long used electrical heating of the bath, keeping the temperature approximately constant by varying the current according to the readings of an ordinary sensitive thermometer. For the present experiments a higher degree of temperature regulation was necessary, and a more elaborate thermostat arrangement has been employed. By means of this the temperature of the bath can be kept constant within 0·001° C. for long periods, thus eliminating, practically completely, any heat-flow in the instrument, and so ensuring that the reading of the galvanometer connected to the thermopile really represents the true rate of heat-production of the tissue lying on the hot junctions. The extreme accuracy of the method of temperature regulation employed, and its relative simplicity, may make it of value for other purposes; for example, it might eliminate the necessity of differential arrangements in volumetric gas measurements, since 0·001° C. at 300° absolute represents only 1 part in 300,000, or one-tenth of a cubic millimetre in 30 c.c. In all the experiments described below, this thermostat was used.

The essential condition for such temperature regulation is that the bath should be extremely well stirred. A small electrically driven Lennox blower was employed, delivering a blast of air through a tube to the bottom of a long cylindrical Dewar flask (10 cm. diameter) which was filled with water, adjusted initially to be 1° to 3° above the temperature of the room. In this water was placed a platinum resistance thermometer, insulated in a glass tube. The resistance thermometer was supplied with balancing leads, so that its readings were independent of the temperature of its connections to the bridge. The latter was of the ordinary kind, except that one of its arms (which were all of low resistance) was connected in parallel with an adjustable high resistance, which acted as an extremely sensitive fine adjustment. A change of 1 ohm in this high resistance represented about 0·001°. The bridge was connected to a sensitive moving-coil galvanometer, read on the same scale as the galvanometer connected to the thermopile; thus the experimenter could readily observe the temperature of the bath, and the speed and direction in which (if at all) it was changing. The temperature was controlled as follows:—

A resistance wire was soldered to copper leads and drawn through a long narrow glass tube; the tube was bent back on itself in the middle and filled with thick paraffin oil. The resistance wire in its glass cover was entirely below the level of the water surface, but the ends of the glass were well above it, so that there was no risk of an electrical leak of warming current to thermopile or resistance thermometer. A current was led from six large accumulators to the resistance wire through a milliammeter and a pair of rheostats, a low resistance and a high resistance rheostat in parallel. The strength of the current was regulated by hand, the high resistance rheostat providing a fine adjustment. By carefully watching for a few minutes the movements of the spot of light from the galvanometer connected to the resistance thermometer, and by making the appropriate adjustments of the warming current, any initial change of temperature of the bath could be checked, and then by observation and readjustment every 3 to 5 minutes the temperature could be maintained constant for long periods within ± 0·001°, *i.e.*, to within ± 5 mm. on the galvanometer scale. The temperature control is relatively simpler when the bath is not much hotter than the room; under ordinary conditions, however, it provides only an inconsiderable addition to the duties of the experimenter. No doubt, by means of a photo-electric relay, it could be made automatic, but without special arrangements such a relay would have to turn the warming current off and on, rather than adjust it in strength, and the success of the system adopted is probably due largely to the continuity of its working. If required, a little extra attention on the part of the observer can readily secure a still greater accuracy of temperature control, say, to 0·0005°, but for the present purpose this was unnecessary; in fact, constancy to within ± 0·003° was sufficient.

D.—*Thermopile.*

Theoretically it should be possible to measure the rate of resting heat-production of a muscle lying on a thermopile by determining the displacement of the galvanometer from its zero when connected to the thermopile. This assumes (*a*) that in the absence of heat-production the hot and cold junctions will settle down finally to the same temperature, and (*b*) that there are no other e.m.fs. in the circuit to cause a displacement. The first condition will be satisfied if the temperature of the bath in which the thermopile chamber is placed remains sufficiently constant, and if no heat can leak in or out along the rods and wires connecting the instrument to the outside; for then, after a sufficient interval, the whole of the system must settle down to a constant temperature. The thermostat arrangement described above, and a sufficient depth of immersion to prevent heat-flow, should be adequate to ensure the realisation of condition (*a*).

In spite, however, of every precaution to maintain a sufficient temperature control, it became obvious, during the present investigation, that no thermopile available would measure the rate of resting heat-production. Usually after temperature equalisation was complete there was a negative deflection from galvanometer zero, suggesting the impossible phenomenon of a steady heat absorption by the resting muscle. This same apparent negative heat-production has for several years been noticed by my colleague Mr. Hartree at Cambridge, but no explanation has been forthcoming. Recently one of Mr. Hartree's thermopiles has been tested in my thermostat; it showed the same negative deflection. Moreover, in many experiments made in the autumn of 1927 with the "brass-collar" thermopile described in 1925 (A. V. Hill (4), figs. 1 and 2, p. 239), with every effort made to secure good insulation and adequate temperature control, not only did negative displacements of the galvanometer occur with a muscle at rest, but also on occasions ridiculously large positive ones, while the change of displacement associated with a replacement of nitrogen by oxygen in the chamber was far too great to be attributed merely to an alteration in the metabolism of the muscle.

It became clear that galvanic effects were at work, that the insulation which we had regarded as adequate was inadequate, and that moisture was able to reach the elements of the thermopile and so set up electrode potentials and galvanic currents. If moisture be in fact capable of penetrating the insulation and making it, to some slight degree, an electrolytic conductor, it is not remarkable that such effects should be produced in the circuit. There is a considerable difference between the electrode potentials of silver and of constantan, and if wires of these two metals be placed in salt water and connected to a galvanometer, a large deflection will result (fig. 1A). Soldering the two wires together diminishes, but does not abolish, this deflection: most of the current may be short-circuited through the junction, but some of it still travels round in the external circuit (fig. 1B). Thus if a thermo-element be placed in a conducting medium, we should expect to find a galvanic effect from it, and when a large number of elements is connected in series, such effects would mount up and might produce a considerable effect. In these thermopiles the insulation was, in fact, fairly good, otherwise the galvanic effect would have been greater than observed. By increasing the efficiency of the insula-

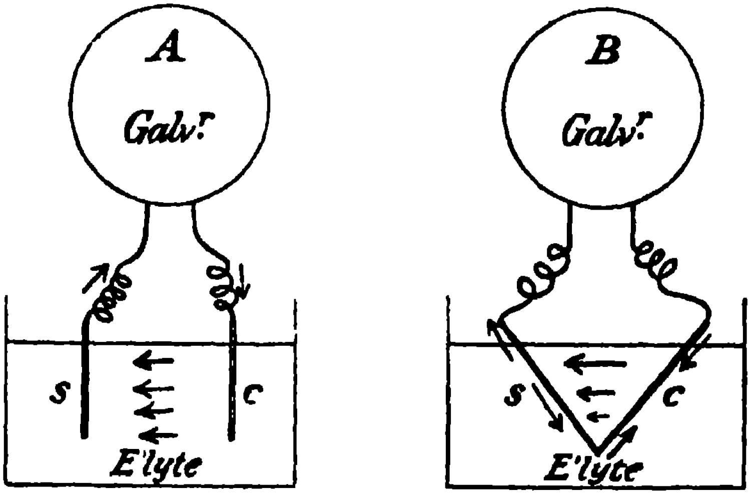

Fig. 1.—Diagram to show the galvanic effect due to a thermocouple of silver-constantan immersed in an electrolyte. Left, elements separate; right, elements joined. *s*, silver; *c*, constantan.

tion, and diminishing its capacity for absorbing moisture and becoming an electrolytic conductor, we might hope to avoid these errors. The success of the new thermopile constructed on these lines indicates that the above explanation of the disturbances observed is correct.

For a measurement of the resting heat-rate it was necessary to avoid the errors described; for other purposes also, where a constant base-line is required over a considerable interval (*e.g.*, in a determination of the recovery heat), it would be a great advantage to be sure that the displacement of the galvanometer really represented heat-production only, uncontaminated by any (perhaps inconstant) error. The thermopile, therefore, which is shown in fig. 2, has been designed and constructed for me by Mr. A. C. Downing. Properly treated it avoids all the errors described; it can measure the resting heat-rate as readily as the heat produced by stimulation; in ease and simplicity of working, and in quickness of response, as well as in accuracy and freedom from errors, it represents far the best instrument at present available for myothermic measurements.

The principles underlying its design were as follows:—

(1) Heat conduction between the different parts of the frame should be as good as possible, in order (*a*) to ensure rapid temperature equalisation, and (*b*) to avoid differences of temperature along the face of the thermopile. A rapid settling down to a constant temperature saves time and helps to avoid artificial differences of temperature between hot and cold junctions. To eliminate temperature differences along the face of the thermopile is essential in experiments where the muscle shortens and work is done; otherwise hotter or colder points come upon the junctions during shortening, and cause unaccountable and often unrealised errors. It is still to some degree doubtful how far any experiments hitherto recorded on the heat-production of muscles allowed actually to shorten are completely free from this objection.

(2) The insulation, while as thin as possible over the hot junctions in order to allow rapid conduction of heat from the muscle, should be very good, and should consist of materials unlikely to absorb appreciable quantities of moisture and so become electrolytic conductors.

(3) The wires should be very thin, and the hot junctions should be imbedded in a thin smooth sheet of insulating material of small heat capacity, not varnished on to a thick sheet of insulator as in the "brass-collar" thermopile previously described. By such means a more rapid response to heat liberated by the muscle, and a higher sensitivity, may be secured.

(4) The cold junctions should be rather distant from the hot ones and in the

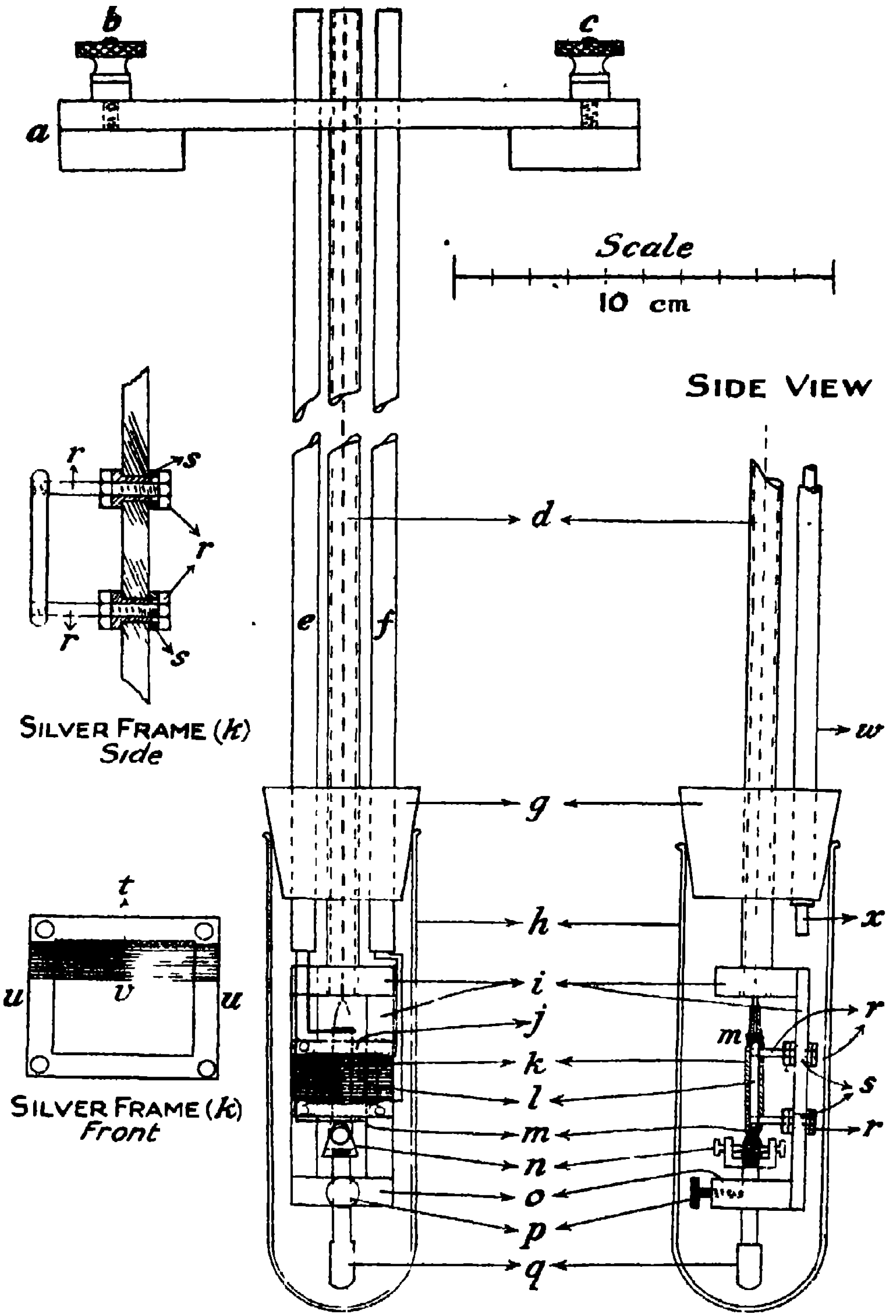

FIG. 2.—"All-metal" thermopile, for a pair of frog's sartorius muscles.—Centre, front view; right, side view; left, enlarged front and side views of silver frame. Scale refers to centre and right only. *a*, vulcanite carrier; *b*, brass terminals to electrodes; *c*, copper terminals to thermopile; *d*, brass tube admitting muscle connection; *e*, glass tube carrying electrode leads; *f*, glass tube carrying thermopile leads; *g*, rubber stopper; *h*, glass cover; *i*, brass carriage; *j*, silver frame; *k*, muscle; *l*, thermopile; *m*, electrodes; *n*, muscle clamp; *o*, brass carriage; *p*, screw; *q*, vulcanite rod; *r*, brass rods and nuts; *s*, "Elo" bushes; *t*, hot junctions; *u*, cold junctions; *v*, wire windings; *w*, gas exit-pipe; *x*, gas inlet-pipe.

immediate neighbourhood of a good heat conductor and of a large heat capacity, in order to ensure that they do not rapidly warm up as the result of heat conducted along the thermo-elements from the muscle on the hot junctions. Otherwise the sensitivity is diminished, especially in respect of a prolonged heat-production, not so much by cooling of the hot junctions as by warming of the cold ones.

These principles have led to the construction of the thermopile shown in fig. 2. As far as possible the carriage and supports of the thermopile are of brass, including the tube holding it and connecting it to the outside. The thermopile is wound on a thinly insulated silver frame, silver being the best heat-conductor practically available. This silver frame is mounted on four brass rods, which are fixed to the carriage through thin insulating bushes. The silver frame and the brass carriage and tube, indeed all metal surfaces inside the muscle chamber, are coated with several layers of " Elo " varnish. This material, a British equivalent of " Bakelite," manufactured by Messrs. Birkby of Liversedge, Yorks, is an artificial resin soluble in alcohol or acetone. It requires heat treatment for some hours at 120° C. after drying ; this process renders it completely insoluble, and very resistant to moisture, as well as providing a smooth and almost glass-hard surface. The insulation was applied with great care ; an alcoholic solution was painted on and allowed to dry ; the object was then placed in an oven and slowly warmed to 120° C. (too rapid warming spoils the surface by causing bubbles) ; it was left in the oven for some hours, until the temperature had fallen to that of the room. Several coats were applied in this way, with heat-treatment after each.

The silver frame, in addition, was fitted with a strip of thin hard paper round its outside vertical edges, to take the pressure of the wires which otherwise may cut through the insulation. This paper was coated with varnish, and heat-treated, making a thin but very strong layer of insulator between the wires and the silver frame. Since the carriage had to be baked the bushes could not be made of vulcanite : heat-treated " Elo " was employed. These bushes were inserted to ensure complete electrical insulation between the thermopile and the carriage, the latter being in electrical contact, through the brass tube, with the water-bath.

The thermopile itself was made by electroplating constantan wire, of diameter 0·152 mm. The wire was wound tightly, and as closely as possible, round the silver frame from end to end ; the hot junctions were arranged to be on both faces down the middle, while the cold junctions were on the outside vertical edges. The wires after winding and electroplating were coated with " Elo " varnish and heat-treated, the process being repeated several times, until the layer of wires gave the impression of being imbedded in a thin solid sheet of glass. After one or two coats, the space inside the frame is completely cut off from the outside by a layer of wires and varnish. If the temperature were now raised to 120° C., the pressure inside would increase by nearly half an atmosphere, which might bulge the wires and crack the insulator. To avoid this contingency a narrow hole was drilled in the silver, to allow the entrance and exit of air, this hole being plugged ultimately with shellac. After the thermopile was completed the four brass rods were screwed into it, and it was bolted to the carriage, which had already been well insulated with "Elo" varnish. The whole instrument was then varnished once again and heat-treated, after which it was ready for connecting up in the manner shown in fig. 2.

The use of a silver frame in this way must go far to ensure equality of temperature at different points of the thermopile; it is difficult to imagine a temperature gradient of appreciable magnitude in such a good conductor as silver. The "Elo" bushes are, of course, poor conductors of heat, but they are very thin, and apart from them the silver frame is in good thermal contact with the carriage and through that with the water in the thermostat. Thus temperature equalisation is relatively rapid. The layer of wires and insulator in the neighbourhood of the hot junctions is very thin and of almost negligible heat capacity; thus the response to a rise of temperature in the muscle is rapid, and the sensitivity is high. On the other hand, the cold junctions are in close thermal contact with, though insulated electrically from, a relatively large block of silver: thus they should remain cool in spite of heat conducted to them from the muscle along the wires. The glass cover was made as narrow as possible, to keep the gas space small and to quicken conduction between thermopile and thermostat.

After the insulation with "Elo" was complete, and the thermopile mounted and connected up, it remained to insulate the leads and to make everything as solid and as water-tight as possible. For this purpose the thermopile itself was touched up with an alcoholic solution of shellac, while the leads were embedded and the electrodes held fast in Chatterton compound melted on with a very small gas jet (about 1½ mm. long) coming from a fine glass tube. To complete the insulation the whole instrument was then dipped in molten paraffin wax (melting point 40° C.), mixed with white beeswax; the same wax was painted thickly over the cold junctions to act as a further heat-capacity, and to shield them from draughts of air, and also over all parts where further protection seemed desirable. Its surface was then melted with the fine gas jet, to ensure continuity and smoothness, and the wax was cleaned off the electrodes. Over the hot junctions the layer of paraffin wax was kept exceedingly thin, but it was very carefully made continuous and smooth, with the small gas jet, before almost every experiment. If this precaution be neglected for several days galvanic effects may become apparent. The use of paraffin wax in this way, on top of "Elo" varnish, provided that care be exercised in melting it on in a thin but continuous layer, seems to render the thermopile completely unaffected by moisture, and immune to galvanic disturbances; immersion for many hours in Ringer's solution has no harmful effect, and the instrument can be trusted to read the resting heat-rate of the muscle with accuracy. That no electrical leaks occur can be shown by connecting an accumulator to the two electrodes and allowing a direct current to run through a dead muscle lying on the thermopile. No deflection of the galvanometer is seen, apart from that due to the heating effect of the current.

The electrodes were of silver wire, the lower one (in the figure) passing between the muscles at their attachment to the bone at the pelvic end, the upper one lying round them, and pulling them together slightly in order to ensure a good contact with the thermopile. The muscle passed over the silver frame at each end, in close contact with it apart from a thin layer of insulator; by this means heat produced in parts of the thermopile not actually in contact with the hot junctions must be largely deflected, thereby eliminating any possible small error due to the fact that in calibration the last few millimetres of the muscle (beyond the upper electrode) were not heated.

The muscle clamp is of the usual type, holding the bone at the pelvic end. The gas inlet pipe is supplied with a rubber tube (not shown) ending in a glass jet at the bottom of the chamber. The gas exit pipe surrounds the inlet pipe and is cut short just below the stopper. When the upper ends of the muscles are just covered the chamber holds about 100 c.c. of Ringer. There are about 100 thermocouples in the thermopile.

E.—Calibration.

For purposes of calibration, and for making control curves, an alternating current is required. There being no A.C. supply in the laboratory at University College (as there is at Cambridge) a valve-generator was employed, giving what is stated by the Cambridge Instrument Company to be a fairly pure sine-wave current. This was used with a frequency of 200 to 500 cycles per second.

It was passed, as usual, through the dead muscle, at the end of an experiment, by the electrodes shown in fig. 2. Its strength was measured by allowing it to flow through an extremely fine insulated resistance-wire, wound non-inductively round the hot junctions of a sensitive thermopile, the hot wire being in series with the muscle. Sartorius muscles only were employed, being sufficiently uniform in cross-section to give practically a uniform rise of temperature under the influence of the warming current.

The muscle was killed by a minute or two of severe over-stimulation ("electrocution") with an induction coil. The strong currents used produce enough heat in the muscle to make it necessary to wait 30 to 45 minutes for the temperature to settle down completely again. This method is far preferable to killing by chloroform; chloroform is apt to harm the insulation of the thermopile (especially if this be a coating of paraffin wax), it may remain in traces in the insulation and injure the muscle in subsequent experiments, and often it leaves the muscle rigid and somewhat altered in form and position on the thermopile. For exact controls the possibility of this last error is serious, and it does not exist in the case of muscles killed by "electrocution," and calibrated not too long afterwards.

Calibration was conducted as follows for the two cases (*a*) of "deflection," and (*b*) of "area." In the former it was desired to express 1 mm. maximum deflection on the scale, representing the "initial" heat, in gram-centimetres of energy; in the latter case to express 1 mm. permanent deflection on the scale, under the influence of a constant warming current, in gram-centimetres per minute. Of all methods hitherto employed for calibration, the present is probably the most convenient and accurate.

(*a*) "*Deflection*" *Calibration.*—Switch S (1) (fig. 3) was turned to connect the valve generator V to the apparatus. Keys K_1 and K_2 on a Lucas revolving contact-breaker (see Parkinson (5)) were adjusted to any suitable interval *t*, and closed in order. Switch S (2) was connected to the muscle electrodes and switch S (3) to the muscle thermopile MT. On releasing the drum of the contact-breaker the alternating current was allowed to pass for time *t* through the muscle and the hot wire in series, and a deflection M was obtained on the galvanometer connected to the muscle thermopile. Switch S (3) was then connected to the hot-wire thermopile HT, everything else remaining unchanged, and the process repeated, a deflection H being obtained on the galvanometer. Switch S (2) was next connected to the resistance box and the current passed again, the resistance being varied in successive observations until the same deflection H was obtained for the hot wire: the resistance R in the box was then equal to that of the muscle. The process was repeated two or three times, to obtain accurate mean values of M, H and R.

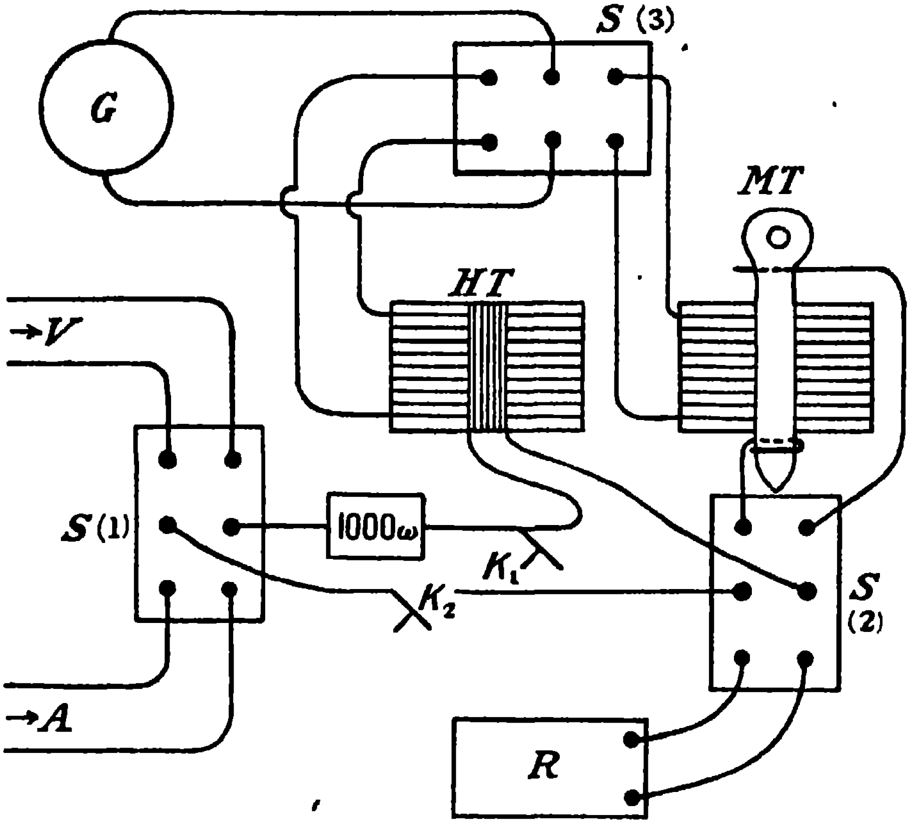

FIG. 3.—Connections of thermopile, for calibration with alternating current. MT, muscle thermopile carrying muscle; HT, hot-wire thermopile carrying hot wire; R, adjustable resistance box; K_1, short-circuit key, and K_2, in-circuit key, of revolving contact-breaker; S (1), S (2), S (3), switches; G, galvanometer; V, valve generator; A, accumulator.

It was now necessary to calibrate the hot wire with a direct current. Switch S (1) was turned to connect the apparatus with an accumulator A of measured e.m.f. *e*. Switch S (2) was connected to the resistance box, a known total resistance *r* being in the circuit (of this, 1000 ω was permanently in the small box shown, which was placed there to protect the battery or valve generator when K_1 and K_2 were closed, and 1768 ω was in the hot wire itself). Switch S (3) was turned to connect with the hot-wire thermopile HT. K_1 and K_2 were then closed, and the constant current *e*/*r* allowed to pass for time *t* seconds through the hot wire, causing a deflection *h* on the galvanometer. This was read two or three times and a mean taken. The value of

1 mm. deflection of the galvanometer, when connected to the muscle thermopile, could then be calculated from the formula

$$1 \text{ mm.} = \frac{\text{HR}}{\text{M}} \times \frac{e^2 t}{r^2 h} \times 1\cdot019 \times 10^4 \text{ gr. cm.}$$

Usually 1 mm. deflection represented about 0·3 gr. cm.

(*b*) "*Area*" *Calibration.*—The whole procedure was similar, except that a weaker alternating current was used and allowed to flow continuously through muscle and hot wire. The galvanometer was read when connected (i) to the muscle thermopile MT, and (ii) to the hot-wire thermopile HT, with no current running. Switch S (1) was connected to the valve generator, switch S (2) to the muscle. K_2 was closed, K_1 left open. After 3 or 4 minutes the galvanometer was read again, a constant deflection M being found when connected to the muscle thermopile and a constant deflection H when connected to the hot-wire thermopile. Switch S (2) was then joined to the resistance box, and the latter adjusted until the deflection of the galvanometer connected to the hot wire was again H : R; the resistance in the box was then the same as that of the muscle.

Calibration with a direct current was similar. Switch S (1) was connected with an accumulator of e.m.f. e, switch S (2) with the resistance box, a total resistance r being introduced into the circuit. Switch S (3) was connected with the hot-wire thermopile. K_2 was then closed, and the constant current allowed to run in the hot wire, causing in a minute or two a permanent deflection h. The value of 1 mm. permanent deflection of the galvanometer, when connected to the muscle thermopile, could then be calculated from the formula

$$1 \text{ mm.} = \frac{\text{HR}}{\text{M}} \times \frac{e^2}{r^2 h} \times 1\cdot019 \times 10^4 \text{ gr. cm. per second.}$$

It is usually convenient to express rates of heat-production in gram centimetres per *minute*, and to evaluate the area of the deflection-time curve in "millimetre-minutes." If this be done the value of 1 "millimetre-minute" is given by the formula,

$$1 \text{ mm.} \times \text{min.} = \frac{\text{HR}}{\text{M}} \times \frac{60e^2}{r^2 h} \times 1\cdot019 \times 10^4 \text{ gr. cm.}$$

Usually 1 mm. × min. represented about 0·7 gr. cm.

F.—*Gases Employed.*

Oxygen or carbon dioxide was run into the muscle chamber from an aspirator, in which the gas stood over water at room temperature. Carbon dioxide was prepared in a Kipp, care being taken to exclude oxygen in collecting it. Cylinder nitrogen was employed, containing about 0·3 per cent. to 0·7 per cent. of oxygen, but before passing to the chamber it was forced through a porcelain filter candle immersed in a strong solution of sodium hydrosulphite ($Na_2S_2O_4$) and soda in an aspirator (see Kautsky and Thiele (6)). The very fine bubbles of gas rising slowly through the liquid have their contained oxygen dissolved before they reach the surface; the gas then passed through four wash-bottles, two containing NaOH solution to dissolve H_2S or SO_2, two containing water to saturate the gas with moisture. The purified damp nitrogen then flowed directly into the muscle chamber. Since nitrogen is slightly lighter than air, and so might gradually rise along the tube containing the thread connecting muscle to tension lever, and thus allow oxygen from the air to enter, it was thought advisable throughout all experiments under anaerobic conditions to maintain a very slow inflow of purified nitrogen. To hinder the diffusion of oxygen through the tubes carrying the nitrogen to the muscle chamber, rubber was replaced as far as possible by lead pipes.

Analysis of the gas passing to the chamber, frequently repeated, invariably showed, with the Haldane apparatus, no measurable amount of oyxgen, certainly less than 0·01 to 0·02 per cent. This method of obtaining an oxygen-free atmosphere in the muscle chamber is very convenient and effective. In order, in certain experiments, to make doubly sure of anaerobic conditions, the purified nitrogen was passed through a wash-bottle containing M/40 NaCN solution neutralised to *p*H 7·0. This gives off traces of prussic acid vapour to the gas passing through it; if a muscle be stimulated in oxygen which has passed through the cyanide solution no recovery heat is observed, so with purified nitrogen, containing less than 0·02 per cent. of oxygen, with cyanide vapour added, we may reasonably assume completely anaerobic conditions.

It is not advisable to employ hydrogen in place of nitrogen. Being so much lighter than air it is more likely to rise in the tube of the thermopile chamber and so allow oxygen to enter. More serious, however, is the fact that its heat conductivity is so much greater than that of air, oxygen or nitrogen that the behaviour of the thermopile is altered by its presence. In the case of a muscle in one of the other gases, nearly all the heat loss appears to be along the thermopile itself, and so obeys simple rules. In hydrogen, however, the loss of heat is largely through the gas, which alters the calibration and introduces unnecessary disturbances. Oxygen and nitrogen have nearly the same heat conductivity; carbon dioxide has a lower one: trials showed, however, no appreciable difference between the calibration numbers in oxygen or nitrogen on the one hand and in carbon dioxide on the other—presumably in either case the loss of heat through the gas is relatively so small that alterations in it have little effect.

Owing to the relatively small amount of heat loss through the gas it is possible, without disturbance, to maintain a slow flow through the chamber throughout an experiment. This was necessary (*a*) when one gas had to replace another *during* a series of observations, *e.g.*, when oxygen was introduced in order to allow recovery to go on after previous stimulation in nitrogen; (*b*) when a gas dissolved in the muscle (*e.g.*, carbon dioxide) had continually to be swept away; and (*c*) when it was required to maintain in the chamber an atmosphere of carbon dioxide or nitrogen completely uncontaminated by air The gas in such cases was allowed to pass slowly into the chamber down a tube in the water bath; it had previously been saturated with moisture at room temperature (1° or 2° below the bath) and must have attained almost complete saturation at the temperature of the muscle by bubbling through a shallow layer of Ringer's solution at the bottom of the chamber as it entered. In any case experience showed that a slow but sufficient rate of flow of the gas could be allowed, without causing any measurable disturbance. A more rapid flow produces considerable errors.

G.—*Phosphate Ringer's Solution.*

It has been found, by experience that the muscles employed (sartorii of *Rana temp.* or *esc.*) behave best and survive longest if, after dissection and mounting on the thermopile, they are soaked for some time, one to three hours, in Ringer's solution before stimulation is begun. To ensure rapid temperature equilibration the Ringer was adjusted as closely as possible to the temperature of the bath, before being introduced into the muscle chamber. Then oxygen was slowly bubbled through it, partly to keep the muscle well oxygenated, partly to secure mixing and quicker attainment of a constant temperature in the chamber. The experiments were all performed in gas, not in Ringer, so the latter was removed after a suitable period of immersion of the muscle, and replaced by nitrogen or oxygen as required. In 20 to 30 minutes after the removal of the Ringer the temperature conditions were usually sufficiently steady to allow the experiment to begin.

Some trouble was experienced during the autumn in getting muscles to survive well on the thermopile. Recent work on the rôle of phosphates in muscle, especially the demonstration by Neugarten (7) of the improvement in performance resulting from the addition of inorganic phosphate to Ringer's solution, suggested that the muscles might be caused to survive better if the Ringer's solution contained phosphate. It was found empirically that an ordinary Ringer's solution, every 100 c.c. of which contained about 10 mgrs. of P in the form of sodium phosphate, adjusted to a *p*H of about 7·2, gave the best results. It is advisable not to use too alkaline a solution of phosphate or the calcium will be precipitated; and it is wise to add the phosphate freshly to the Ringer's solution before use, for the same reason. Even at neutrality the calcium is precipitated from phosphate Ringer in a few days. Considerable improvement has resulted from this use of phosphate, and it has been made a routine. A stock solution of sodium phosphate, adjusted to *p*H 7·2, was prepared, containing 500 mgrs. of P per 100 c.c.; 2 c.c. of this were added to 100 c.c. of Ringer immediately before use. Experiments shortly to be published by Parkinson confirm and extend the conclusions of Neugarten in respect of the beneficial effect of including phosphate in the Ringer.

During the course of these experiments an investigation was started by Stella, which throws light on the improvement due to phosphate, and will shortly be published. Stella has shown, by studying the diffusion of inorganic phosphate into and out of frogs' muscles, that the free inorganic phosphate in the resting muscle fibre is in diffusion equilibrium with a Ringer's solution containing phosphate to the extent of about 8 mgrs. per cent. P. If less phosphate than this be present in the Ringer some diffuses out; if more, then some diffuses in. The rest of the phosphates inside the muscle fibre are in a combined form, incapable of diffusing. Phosphagen (Eggleton and Eggleton (8)) and hexose phosphates make up a large part of this combined phosphorus, but probably not all. Presumably the free inorganic phosphate content of the living muscle is adjusted to an optimum level, and if the concentration inside the fibre be allowed to fall by the use of a solution containing little or no phosphate, the condition of the surviving muscle depreciates more rapidly than under more normal conditions. The solution arrived at empirically as the best agrees approximately with that which would be deduced from Stella's diffusion experiments.

H.—*Other Experimental Details.*

(i) *Muscles Employed.*—In all cases a pair of sartorius muscles was used, from *Rana esc.* or *Rana temp.* These have the advantages (*a*) of being approximately straight fibred and of uniform cross-section, and (*b*) of being thin enough to ensure (i) rapid temperature equilibration with the thermopile, and (ii) rapid diffusion of gases in or out.

(ii) *Stimulation.*—For series of single break shocks uniformly spaced the revolving cam contact-breaker described by Gerard, Hill and Zotterman (9) was used, driven at the required speed (20 to 40 shocks per minute) by a motor. A drum on which the twitches were recorded was driven by the same motor. For a tetanus an ordinary Harvard coil was employed, the duration being determined by a Lucas revolving contact-breaker. The stimuli were always maximal, but not supermaximal, and never so strong as to cause any appreciable heat-production in the dead muscle.

(iii) *Insulation.*—To avoid possible disturbances due to electric leaks, all the apparatus was extremely carefully insulated from the ceiling, walls and floor by means of vulcanite and paraffin wax. Wires were held by paraffined string, and the tables were supported on vulcanite blocks. The observer stood on a wooden platform insulated from the floor by vulcanite.

(iv) *Mechanical Response.*—The tension-lever, placed immediately above the muscle chamber, was a light one mounted on a Palmer stand. It gave 1 mm. deflection for 3·67 grams., and had a short period. It was connected to the muscle by a thick linen thread. The initial tension was adjusted by trial in most cases to give the greatest response to a single shock (see A. V. Hill (4)); it was of the order of a few grams.

(v) *Control.*—An adjustable potential divider with a reversing key was employed, when necessary, to supply a small current in the galvanometer circuit in order to adjust the spot of light to a suitable position on the scale. A special resistance box with copper terminals and manganin coils was used, by which any required resistance could be introduced into the galvanometer circuit; and another suitable box, also with copper terminals and manganin coils, could be employed when required as a shunt. No detectable e.m.f. was present in the circuit, other than in the thermopile, as was shown by the fact that reversing the current from the latter exactly reversed the deflection of the galvanometer.

Summary.

Improvements in myothermic apparatus have made it possible to measure, with relative accuracy, in the sartorius of the frog or any similar muscle, not only the heat suddenly produced by a single stimulus, but that liberated over long intervals at rest, or in recovery, or as the result of prolonged discontinuous stimulation. The essential factors are:—

(*a*) A moving-coil galvanometer of high sensitivity and short period.

(*b*) A method of measuring total heat from the area of the deflection-time curve.

(*c*) A thermostat in which the temperature is maintained constant within 0·001° C. for long periods.

(*d*) An "all-metal" thermopile, which responds quickly, settles down rapidly, and is very completely insulated.

Other details are described, including :—

(*e*) An improved method of calibration.

(*f*) The preparation and use of the gases employed (N_2, O_2, and CO_2).

(*g*) The advantage resulting from a Ringer's solution containing phosphate (7 to 15 mgrs. per cent. P).

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The Rôle of Oxidation in maintaining the Dynamic Equilibrium of the Muscle Cell.

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1. *Experimental.*

It is possible by the methods described in the preceding paper to determine directly, as a deflection on the galvanometer scale, the rate at which heat is being liberated by a muscle at rest on a thermopile. In all that follows this resting heat-rate will be expressed (unless otherwise stated) in grm.-cms. of energy per gramme of muscle per minute, the muscle being weighed, after removing adherent moisture by filter-paper, at the end of an experiment. These units can be converted to grm.-calories per gramme per minute by dividing by 42,400, or to grm.-calories per gramme per hour by dividing by 707. Absolute quantities of heat will be expressed in grm.-cms. per gramme of muscle: these can be reduced to grm.-calories per gramme of muscle by dividing by 42,400.

In every case the sartorius muscles of a frog were used: *Rana temporaria* and *R. esculenta* were employed indiscriminately, no difference being found between them. After careful dissection the muscles were mounted on the thermopile and immersed for 1 to 3 hours in Ringer's solution made with glass-distilled water, and containing sodium phosphate (pH 7·2, 7 to 15 mgs. per cent. P, see preceding paper). In most cases oxygen was bubbled through the solution, to assist in temperature equalisation, to keep the muscle well oxygenated, and to allow it to recover from stimulation necessarily occurring during dissection.

2. *The Resting Heat-Rate of a Muscle in Oxygen.*

On removing the Ringer and replacing it by oxygen, only about 20 minutes are required for the temperature to settle down sufficiently for readings of the resting heat-rate to begin. Contrary to expectation, however, the heat-rate in oxygen of a muscle in good condition (as shown by subsequent capacity to respond to stimulation) is not constant, but continually diminishes (at a decreasing rate) over a long period. It may be several hours before an approximately constant reading is attained. This is not due to oxidative recovery from previous exertion, for it is readily shown that the extra heat-production following stimulation is complete in 20 minutes or so. If, during this phase of diminishing heat-rate in oxygen, the muscle be given a tetanus or a series of shocks, and then allowed to recover, the resting heat-rate will usually be found to have returned to a lower value than obtained before stimulation ; indeed, if anything, stimulation followed by recovery tends to quicken the fall to a constant level. The following examples illustrate this point, which will be seen later to be important.

Table I.—Resting Heat-rate in Oxygen : Effect of Stimulation and Recovery.

***Experiment** of* 2.1.28.—16·2° C. Dissected 10.45 a.m. In oxygenated phosphate-Ringer at 11.15 a.m. At 11.25 a.m. 44 twitches and at 12.7 p.m. 50 twitches, when the Ringer's solution was replaced by oxygen At 1.11 p.m. resting heat-rate *218* : 31 shocks given, liberating total heat (initial + recovery) 6350 : at 1.37 p.m. resting heat-rate *187* : 31 shocks given, total heat 6350 : at 2.3 p.m. resting heat-rate *148*.

***Experiment** of* 22.2.28.—15·6° C. Ringer replaced by O_2 at 11.28 a.m. At 12.39 p.m. resting heat-rate *164* : 62 shocks and 33 minutes' recovery, resting heat-rate *135* : 62 shocks and 40 minutes' recovery, resting heat-rate *115*.

***Experiment** of* 20.2.28.—15·2° C. Ringer replaced by O_2 at 10.53 a.m. At 11.13 a.m. resting heat-rate *353* : 62 shocks and 27 minutes' recovery, resting heat-rate *292*.

***Experiment** of* 13.1.28.—18·6° C. Muscle in oxygenated Ringer 45 minutes, then in oxygen. After 35 minutes, resting heat-rate *43 mm.* on scale (no calibration) : 0·75 sec. maximal tetanus and 17 minutes' recovery, resting heat-rate *40 mm.* : 0·75 sec. tetanus and 18 minutes' recovery, resting heat-rate *35 mm* : 0·75 sec. tetanus and 17 minutes recovery, resting heat-rate *31 mm.*

The absolute value of the resting heat-rate in oxygen cannot be exactly specified, but an approximate number obtained as the mean of 8 observations on 8 good muscles, at an average temperature of 17·6° C. is 180. The actual readings were as follows :—

13·2°	94	19·2°	198
15·2°	292	20·5°	145
15·6°	115	20·5°	190
16·2°	148	20·7°	242

The variation is rather wide, and it is possible that slight injury inevitable to separation from the animal and mounting on the thermopile, however carefully the procedure be carried out, may have increased the metabolism somewhat, more in some cases, less in others. Values of 160 at 20° C. and 100 at 15° C. would seem reasonable minima. Assuming that this heat comes from the oxidation of glycogen, or similar material, in which case we may calculate that

1 grm.-cm. of energy set free is the equivalent of $4 \cdot 59 \times 10^{-6}$ c.c. of oxygen used, these quantities of heat correspond to the following oxygen consumptions :—

15° C.	0·00046 c.c. per gramme per minute.
20° C.	0·00078 c.c. " "

According to Meyerhof and Schulz ((1), p. 559), the oxygen consumption of a resting muscle at 15° C. lies between ·15 and 35 cubic mm. per gramme per hour—*i.e.*, between 0·00025 and 0·0006 c.c. per gramme per minute. The value calculated above for 15° C. lies well within their limits. According to Gerard (2), the oxygen consumption of frogs' nerves averages at 14° C. about 0·00035 c.c. per gramme per minute, rather less, but still of the same order of size. It is clear that in muscle the resting heat-rate in oxygen is sufficiently accounted for by oxidation.

These experiments on the resting heat-rate in oxygen are, in and for themselves, of no great interest. They are, however, essential in the discussion which follows of the resting heat-rate in the absence of oxygen. The reason for the gradual decrease in the heat-rate to a low constant level is not obvious : possibly, after removal from the animal, some substance previously supplied by the circulating blood, and accelerating oxidation, is gradually used up : possibly the change from normal to less normal conditions provokes at first an alteration in the impermeability of the muscle interfaces, analogous to that found to occur (see below) as the result of anaerobic stimulation, which is only gradually restored during prolonged survival in oxygen : possibly even slight injury, inevitable to separation from the animal, may produce changes which only gradually pass off in oxygen. There is no evidence at present as to the cause of this gradual fall in the " metabolism " of the resting muscle, so it is useless to speculate further. The essential points for the later discussion are :—

(i) That the resting heat-rate in oxygen is sufficiently accounted for by oxidation ; and

(ii) That survival in oxygen, and stimulation followed by recovery in oxygen, tend to decrease and certainly do not increase the resting heat-rate.

3. *The Minimum Resting Heat-Rate of a Muscle deprived of Oxygen.*

A muscle is placed on a thermopile and soaked for some time in oxygenated phosphate-Ringer, as described above, the Ringer being then replaced by oxygen. After temperature equalisation has been reached the oxygen is replaced by pure nitrogen, and the resting heat-rate observed. At first there is no change from the value obtaining in oxygen: it takes 15 minutes or more, depending on the thickness of the muscle and its temperature, for all its contained oxygen to diffuse out, or to be used in oxidation. Finally, however, a distinct change sets in, the galvanometer deflection diminishing rather rapidly to about half its previous value. *At this reduced value it remains constant for a long time, with no sign of further decrease.* This deflection, reached after half an hour or so in pure nitrogen, is taken as giving the minimum resting heat-rate under anaerobic conditions. No stimulation beyond a few shocks is allowable, otherwise, as will be seen below, the observation will be vitiated. Twenty-one observations on 21 different muscles, of the minimum resting heat-rate in pure nitrogen (or nitrogen containing HCN vapour) gave the following results :—

Temperature: °C.	13·2	15·2	15·6	17·2	18	18·3	18·6	18·7	18·8	19	19
Heat-rate	26	37	55	65	85	84	107	41	60	60	44
Temperature °C.	19	19·2	19·2	20	20	20·3	20·4	20·5	20·5	20·7	
Heat-rate .	45	80	57	57	123	68	97	111	57	61	

The numbers are somewhat variable, as in the case of oxygen, probably for the same reason. Slight injury, inevitable to separation from the body, may have provoked irreversible changes of some kind, more in some cases, less in others. Values of 65 at 20° C. and 40 at 15° C. would seem reasonable minima. Now it is commonly assumed that an isolated muscle, or other tissue, suddenly deprived of oxygen continues its breakdown unchanged, merely failing to complete its cycle by the oxidative removal, or restoration, of the products of its metabolism. The primary metabolic process in muscle is the formation of lactic acid, and if oxygen be available the lactic acid formed is restored as glycogen, the energy for this endothermic process coming from independent oxidation; if oxidation be not possible, the lactic acid merely accumulates, its rate of formation being, at any rate at first, unchanged.

The evidence for this view is strong; it is the basis of all the extremely important work of Warburg on the metabolism of tumour tissue (3), and in one form or another of much of the work of Meyerhof and the present writer on the energy exchanges of muscle. If we adopt it we should regard the minimum resting heat-rate in nitrogen as representing the anaerobic part of a cycle, of which the final resting heat-rate in oxygen represents the whole. The ratio of the latter to the former, using the 15° C. figures given above, is 100 : 40, *i.e.*, 2·5 : 1; in muscle, according to the latest determination of Furusawa and

Hartree ((4), p. 208), the total oxidative heat bears to the total anaerobic heat due to stimulation a ratio of 2·50 : 1·12, *i.e.*, of 2·23 : 1. The similarity of these two ratios makes it clear that the minimum resting heat-rate under anaerobic conditions can be sufficiently accounted for on the hypothesis that it represents the anaerobic part of the breakdown of which the resting heat-rate in oxygen represents the whole cycle.

If the minimum resting heat-rate in nitrogen be due to lactic acid formation, we may calculate the lactic acid from the heat. According to Meyerhof's latest statement ((5), p. 106), the formation of 1 gramme of lactic acid in muscle is accompanied by the liberation of 380 to 390 calories. Taking 385, 1 gramme of lactic acid would be equivalent to $1 \cdot 63 \times 10^7$ grm.-cms. of energy, or 1 grm.-cm. to $6 \cdot 14 \times 10^{-8}$ gramme of lactic acid. Thus an anaerobic resting heat-rate of 40 at 15° C. would correspond to $2 \cdot 46 \times 10^{-6}$ gramme of lactic acid per gramme per minute, or of 0·0147 per cent. per hour. At this rate it would take over 34 hours to attain the 0·5 per cent. of lactic acid typical of rigor.

4. *The Increment produced by Stimulation in the Anaerobic Resting Heat-Rate.*

If a muscle showing a normal resting heat-rate in nitrogen be stimulated by a maximal (but not super-maximal) tetanus, or by a succession of maximal shocks, the galvanometer, after its usual excursion recording the heat liberated in activity, returns, not to its previous position but to a new one, showing an enhanced resting heat-rate. So long as it remains in nitrogen this increase in the resting heat-rate is maintained : one may continue observation indefinitely, but the galvanometer never returns to its original position. Fig. 1 (top record) shows the isometric response to 183 maximal break shocks delivered

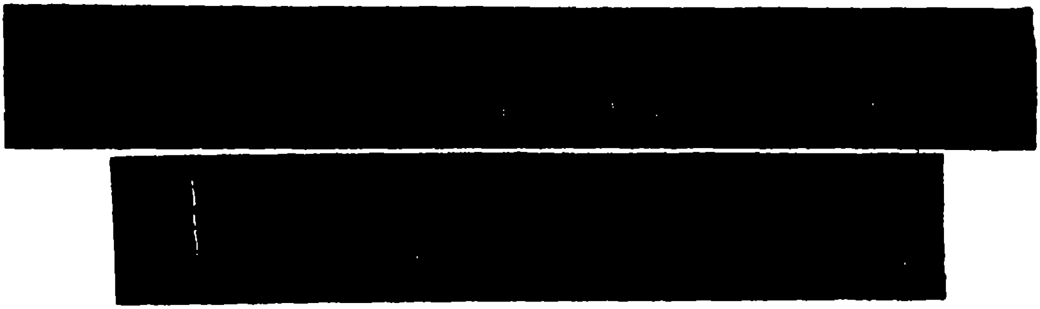

Fig. 1.—Experiment of 30.12.27. Top, Series I, 183 maximal break-shocks in nitrogen, isometric record. Bottom, left, after recovery, Series II, 90 maximal break-shocks in oxygen. Bottom, right, after recovery, Series III, 30 maximal break-shocks in oxygen. Read from left to right. For Series II and III, see Section 10 below.

in nitrogen at 13·2° C. at intervals of 3 seconds: fig. 2 shows the galvanometer deflection resulting from the stimulation. In the latter, every observation made on the scale is plotted, and the total heat can be calculated, as described in the previous paper, from the area of the deflection-time curve.

It will be seen that the curve reaches a maximum at, or near, the moment when the mechanical response is a maximum, and then declines as fatigue comes on: at the end of 6 minutes, when stimulation finished, the curve falls rapidly, and in 5 or 6 minutes the deflection becomes constant again, *but at a new level which is maintained indefinitely thereafter;* the final displacement from the original position (not the galvanometer-zero) being 22 mm. on the scale represents an increment in the resting heat-rate of 119. The original heat-rate was only 26, the muscle being in very good condition and at rather a low temperature: *thus as the result of* 183 *maximal twitches the resting heat-rate has been increased more than five times.*

In such a case as this the base-line from which the heat due to activity is to be reckoned obviously needs consideration. It has been assumed in all that follows that the base-line changes uniformly during stimulation: a horizontal line is drawn backwards through the final level reached, till it cuts the vertical line representing the end of the stimulus: the point so obtained is then joined by a straight line to the origin representing the commencement of stimulation. The broken line so drawn is regarded as the base from which to measure the area of the deflection-time curve. In the case shown in fig. 2, the area is

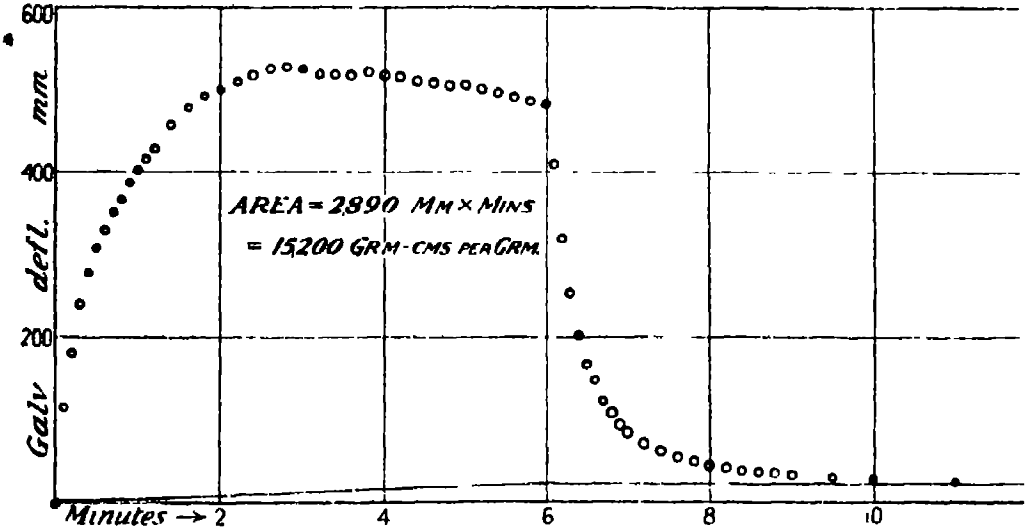

FIG. 2.—Experiment of 30.12.27. Heat record in nitrogen of Series I, fig. 1. Actual observations on galvanometer scale. For base line, see text. Note: the horizontal axis represents the initial resting heat-rate, not the galvanometer-zero.

2,890 mm.-minutes, which, from the calibration, is calculated to be 15,200 grm.-cms. per gramme of muscle. It will be seen from fig. 2 how smooth a deflection-time curve is obtained, and how accurately its area, and therefore the heat, can be calculated. Fig. 2 is typical of all the experiments made.

The process can be repeated several times until the muscle is exhausted. In fig. 3 the heat produced by stimulation is omitted from the diagram, but given numerically. The initial heat-rate in nitrogen was *86* : 64 maximal shocks, liberating total heat 7,830, increased it to *181* : then 98 maximal shocks, liberating heat 12,200, raised it to *287* : finally a further 98 shocks producing heat 8,000 and almost complete fatigue, brought it to *381*. After that the muscle was left for a further six hours, during which the resting heat-rate increased at first somewhat, and then remained more or less constant. The total heat produced by stimulation was 29,100 (0·686 cal. per gramme), while the total heat due to the resting heat-rate was as follows :—

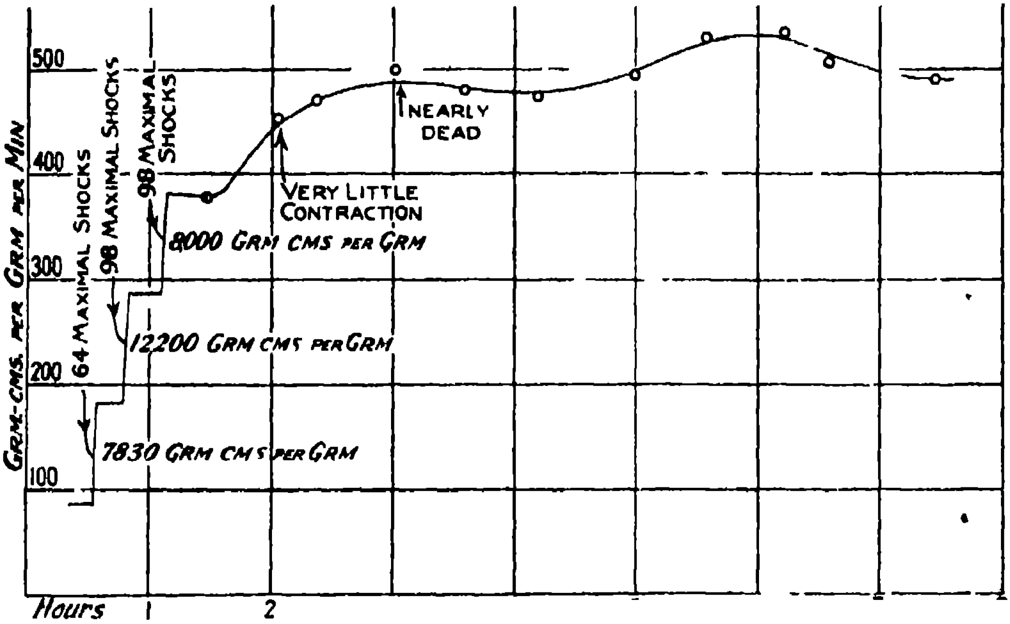

FIG. 3.—Resting heat-rate in cyanide-nitrogen, of muscle stimulated as shown. Experiment of 6.2.28 at 18·3° C.

Up to the end of stimulation..	12,200
From then to the end of the 2nd hour	20,000
2nd to 3rd hour ..	28,500
3rd to 8th hour	150,000
Total	210,700 (4·97 cals. per gramme.)

Fig. 4 shows a similar experiment and explains itself. At the end of the 5th series the muscle was "electrocuted," a process which prohibits observation

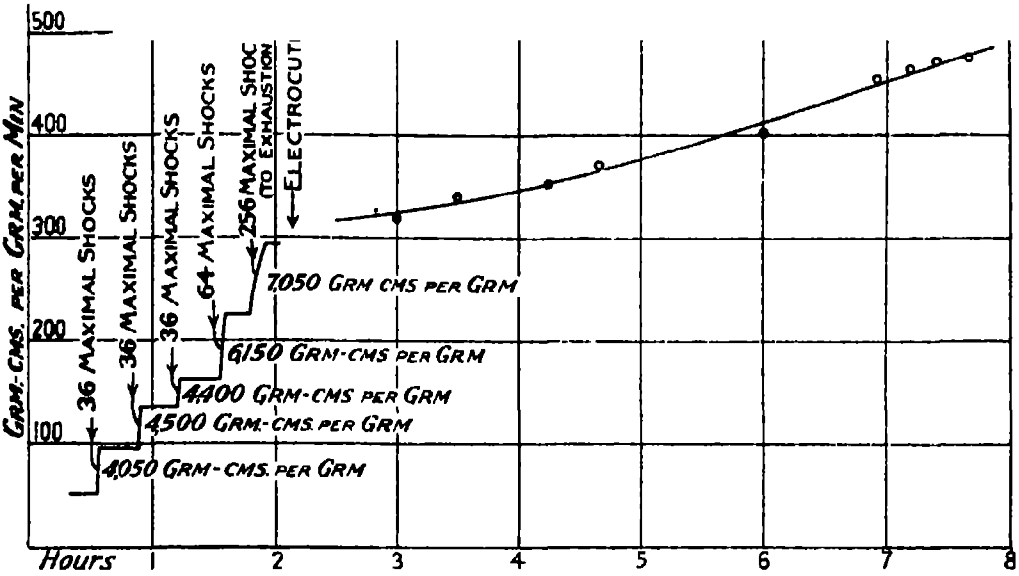

FIG. 4.—Resting heat-rate in cyanide-nitrogen, of muscle stimulated, and "electrocuted" as shown. Experiment of 25.1.28 at 19·2° C.

for a time owing to the temperature disturbance produced by the strong tetanus necessary. After the "electrocution" the resting heat-rate went on increasing steadily, and was still rising at 8 hours. The total heat produced by stimulation was 27,000 (0·637 cal. per gramme), while the total heat due to the resting heat-rate was as follows:—

Up to the end of stimulation	16,440
3rd hour	18,500
4th hour	20,000
5th hour	21,500
6th hour	23,500
7th hour	26,000
8th hour	28,000
Total	153,940 (3·63 cals. per gramme.)

Experiments of this kind have been repeated many times, and the phenomenon always recurs. The greater the amount of heat liberated by stimulation, the greater the increment of the resting heat-rate. There is, in fact, a linear relation between the two quantities. If the resting heat-rate be plotted against the total heat liberated by stimulation, a good straight line is obtained, as shown in fig. 5. The two upper lines in the figure represent the experiments of figs. 3 and 4 respectively (the last point of each was interpolated from a more distant point to keep it within the diagram). The slope of the line is greater, in general, the higher be the temperature (see below), though individual variations of slope occur. The experiments of fig. 5 were made with successive series of separate break-shocks, in the manner described in referring to figs. 1 and 2 above. The same phenomenon occurs, as might be expected, when tetanic stimuli are used (see fig. 6, which gives as exact a linear relation as could be desired). Thus the *increment in anaerobic resting heat-rate is directly proportional to the total heat produced by stimulation.*

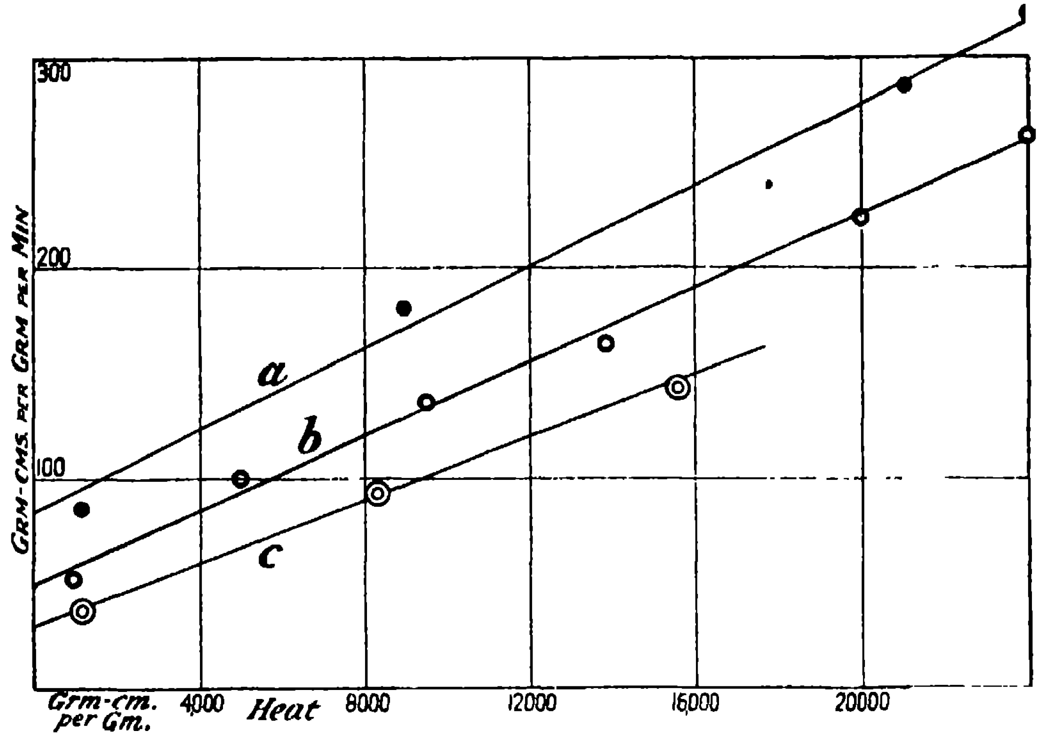

Fig. 5.—Linear relation between anaerobic resting heat-rate and total heat liberated by stimulation in successive series of twitches: three different experiments. (*a*) 6.2.28 at 18·3° C. in cyanide-nitrogen; (*b*) 25.1.28 at 19·2° C, ditto; (*c*) 20.12.27 at 15·2° C. in nitrogen.

The constant factor of this proportion has been determined in a large number of experiments at various temperatures. It will be shown below that the anaerobic resting heat-rate has a large temperature coefficient, of the order of 2·7 for 10° C. Applying this coefficient, the factor has been "reduced" to

20° C. The procedure was as follows: the heat-rate observed, after various degrees of anaerobic stimulation, in any given experiment, was plotted against the total heat liberated by the stimulus. The slope of the line gave the factor K required in the formula

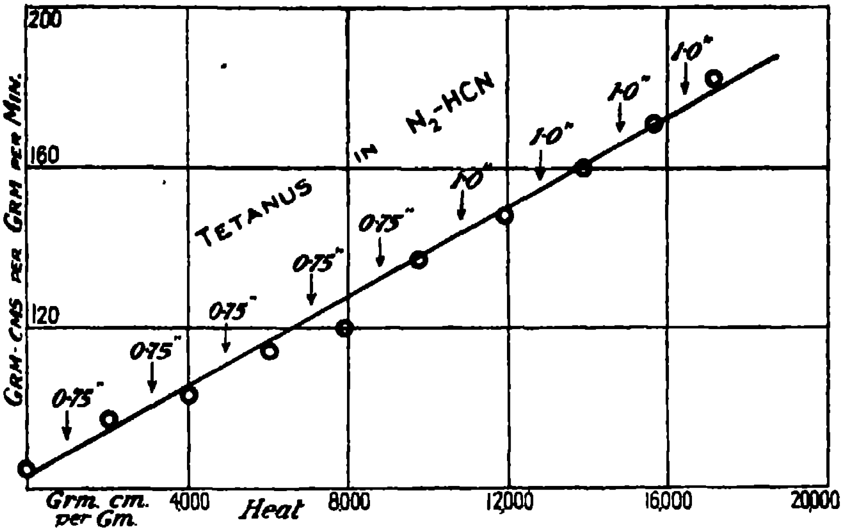

FIG. 6.—Linear relation between anaerobic resting heat-rate and total heat liberated by a succession of tetanic stimuli at 10-minute intervals. Experiment of 16.1.28 at 18° C. Tetanus duration in seconds.

(Increment in heat-rate) = K × (Total heat by stimulation)
grm.-cms. per gramme per minute. grm.-cms. per gramme.

Of 23 determinations made in 23 different experiments, one only was eliminated as giving an abnormally high result: the rest, after "reduction" to 20° C., gave a mean value of K = 0·0122, with an average variation from the mean of 0·0024. These experiments are complex and lengthy; each takes a whole day to complete and several hours to calculate, and involves many delicate measurements and adjustments: the muscles used are variable and sometimes unreliable. In view of this, the fact that in 23 experiments, discarding only one, the average variation of a single reading of K is only 20 per cent. of the mean value is surely a sign that in the above relation we have an expression of some very constant characteristic of muscle tissue.

The results described are novel and somewhat astonishing. Before proceeding further, therefore, it is desirable to examine, as critically as possible, the experiments on which they are based. The phenomenon was first observed when employing cylinder nitrogen, which was found to contain 0·7 per cent. of oxygen. It seemed possible that this oxygen might allow an oxidative recovery process to occur for long periods after stimulation, which would account for the increment in heat-rate observed: the greater the amount of lactic acid formed by stimulation the more rapidly the recovery process might proceed. To eliminate this possibility purified nitrogen was employed (see preceding paper) containing less than 0·01 or 0·02 per cent. of oxygen. The phenomenon occurred exactly as before.

It still seemed conceivable that even the traces of oxygen remaining might produce the effect. It can, indeed, be calculated that at a pressure of 0·02 per cent. of an atmosphere oxygen cannot diffuse more than a few thousandths of a millimetre into a muscle at a speed great enough to account for the heat-rate observed: nevertheless lactic acid might diffuse up to meet it from distant points, and oxidation might occur in the surface layer of the muscle: this oxidation would proceed at a rate proportional to the speed of diffusion outwards of the lactic acid, which would be proportional to its concentration, so that a linear relation between heat-rate and lactic acid formed might obtain.

To eliminate this remaining possibility, cyanide vapour was employed, the nitrogen (see preceding paper) being bubbled through a neutral solution of sodium cyanide before admission to the muscle chamber. In order to make sure that the cyanide vapour employed does, in fact, prevent oxidative recovery, various observations have been made on muscles in oxygen treated with cyanide in precisely the same way as the nitrogen in the preceding experiments. No trace of recovery heat has been observed.

In one experiment, in a series of twitches, the total heat (for a given tension development) was found to be 2·07 times as great in oxygen as in oxygen-cyanide: in another experiment 2·12 times as great: the ratio is the same as for oxygen and nitrogen respectively. Moreover, the galvanometer deflection in cyanide-oxygen follows precisely the same course as in pure nitrogen, returning to zero in 3 or 4 minutes; whereas, in pure oxygen, the recovery heat maintains the deflection for a much longer period. If, therefore, the presence of cyanide so completely eliminates oxidative recovery, even in oxygen, it seems inconceivable that in cyanide-nitrogen mere traces of oxygen could exert any perceptible effect. The phenomenon, however, persisted unchanged in the purest nitrogen containing cyanide: indeed, most of the experiments described above have been made under such conditions. We may safely conclude, therefore, that the *increment of heat-rate after stimulation is not due in any way to oxidation.*

Could it be caused by any physical defect of the instruments employed? Might the increasing acidity of the muscle due to stimulation somehow exert a galvanic effect upon the wires of the thermopile, penetrating their insulation, and so produce a deflection proportional to the change of hydrogen ion concentration?

This possibility was examined in various ways. The muscles were made more acid by filling the thermopile chamber with pure carbon dioxide: in general (see below) the deflection was decreased, and not increased, as a result. A 2-volt accumulator was connected directly to the electrodes: no electric leak was observed, the only effect being that due to a gradual heating of the muscle by the current. Finally (see below) the temperature of the thermostat containing the thermopile-chamber and muscle was lowered from 20° C. to 0° C. A large permanent deflection previously produced by stimulation was reduced 7 to 8 times, but re-appeared when the system was warmed up again. It is very unlikely that any physical disturbance could be so largely affected by a change of temperature, whereas if the increment in heat-rate be due to chemical reactions proceeding within the muscle, we should expect to find just such an effect.

It is clear, therefore, that *the phenomenon is not due to any physical defect in the thermopile.*

Could it be due to injury of the muscle caused by excessive stimuli? It is well known, since the work of Meyerhof and Lohmann (6), that stimulation by too strong a current may cause an after-production of lactic acid, and Furusawa and Hartree (4) proved that too powerful a tetanus may cause a prolonged liberation of heat in a muscle under anaerobic conditions. Moreover, the present experiments have shown that " electrocution " is a very convenient way of killing a muscle, and that after " electrocution " (even of an inexcitable muscle) there may be a large increment in the resting heat-rate. The best answer to this objection is the fact, described above, that the same identical stimuli, applied to a muscle in oxygen, cause rather a decrease than an increase in the resting heat-rate. Moreover, the increment in resting heat-rate produced by not too long anaerobic stimulation may be reversed, as will be shown below, by subsequent recovery in oxygen. It seems unlikely that injury produced by excessive stimuli could be so reversed. Finally, it will be shown later that prolonged anaerobic survival, without stimulation, produces exactly the same effect. We may conclude, therefore, that *the phenomenon is not due to injury caused by the stimuli employed.* We must regard it as a genuine effect, due to properties of the muscle itself.

5. *The Effect of "Electrocution" on the Anaerobic Resting Heat-Rate.*

As pointed out in the preceding paper, "electrocution" by excessively strong induction currents is a much more convenient way of killing the muscle for calibration purposes than the use of chloroform. It has been found that the maximum anaerobic resting heat-rate attainable after ordinary stimulation can be considerably surpassed as the result of "electrocution." For example, the heat-rate in a muscle at 18·8° C. tetanised to an advanced stage of fatigue in cyanide-nitrogen was *345*, but two hours after "electrocution" it was *725*. Again, in an exhausted muscle in cyanide-nitrogen at 18·6° C. it was *670*, whereas after "electrocution" it was *840*. In a muscle nearly inexcitable at 20·5° C. it was *218*: after "electrocution" it was *747*. The five highest values ever found in an "electrocuted" muscle were 840, 840, 890, 1060 and 1176, all in the neighbourhood of 19° or 20° C. The five highest values in a muscle stimulated to complete exhaustion, but not "electrocuted," were 417, 458, 482, 670 and 752, at the same temperature. Such values are not a passing phenomenon, but may be maintained for several, often for many, hours—indeed, in the exhausted muscle the heat-rate tends to increase. The cause of the high values produced by "electrocution" will be discussed below.

6. *Absolute Values and their Consequence.*

A heat-rate of 1000 means 60,000 grm.-cms. of energy (or 1·42 calorie) liberated per gramme per hour. If this were due to lactic acid formation there would be $\frac{1\cdot42}{385} \times 100$, *i.e.*, about 0·37 per cent. of lactic acid formed per hour, or not far short of the lactic acid maximum. Such a heat-rate may last for many hours, so that it cannot be due to lactic acid formation. In the experiment shown in fig. 3, the total heat in 8 hours due to the anaerobic resting heat-rate was 4·97 calories per gramme, while in the experiment of fig. 4, in the same time, it was 3·63: these would correspond to 1·29 and 0·94 per cent. respectively of lactic acid, unheard-of values. It will be seen, moreover, that the heat-rate at 8 hours shows no signs of falling off: it would, in fact, continue for many hours at the same level. The following experiment may be quoted:—

24.2.28.	19° C. in N_2, ½ hour in N_2, heat-rate	60
	8 tetani, heat 12,000 ,,	320
	Electrocuted. 1 hour later ,,	755
	2½ hours after electrocution	840
25.2.28.	17·2° C.—19 hours after electrocution, heat-rate	705
	22 ,, ,, ,, ,,	670
	24 ,, ,, ,, ,,	645
26.2.28.	15·6° C.—44 ,, ,, ,, ,,	425
27.2.28.	19° C.—68 ,, ,, ,, ,,	525

During the whole of this experiment purified nitrogen was running slowly through the chamber: at the end the muscle had a very slight "fishy" smell.

In this experiment, in 68 hours about 55 calories per gramme of muscle were liberated as a result of the resting heat-rate. During the major portion of it the muscle was inexcitable. Too much attention need not be paid to the absolute value, since undoubtedly bacterial processes were at work in the end. There is no reason, however, to suppose that bacterial decomposition of any appreciable extent occurred during the first 24 hours ; a muscle in oxygen may remain normally active for considerably longer than that : yet during 24 hours nearly half the heat was liberated—15 times as much as could possibly be accounted for by lactic acid formation. It is clear, therefore, that *the increment produced by stimulation, or by "electrocution," in the anaerobic resting heat-rate is not due simply, or mainly, to an enhanced formation of lactic acid ;* it must be attributed to anaerobic reactions possessing much more energy than that.

7. *The Increment in Resting Heat-Rate due to Anaerobic Survival without Stimulation.*

If the increment in resting heat-rate be caused either by lactic acid formation or by increasing oxygen want, or by some other accompaniment of activity, we might reasonably expect prolonged survival at rest without oxygen to have the same effect ; there is strong reason to believe that the energy exchanges of activity and of resting survival are derived from the same source. The experiment illustrated in fig. 7 shows that this is, in fact, the case. A muscle

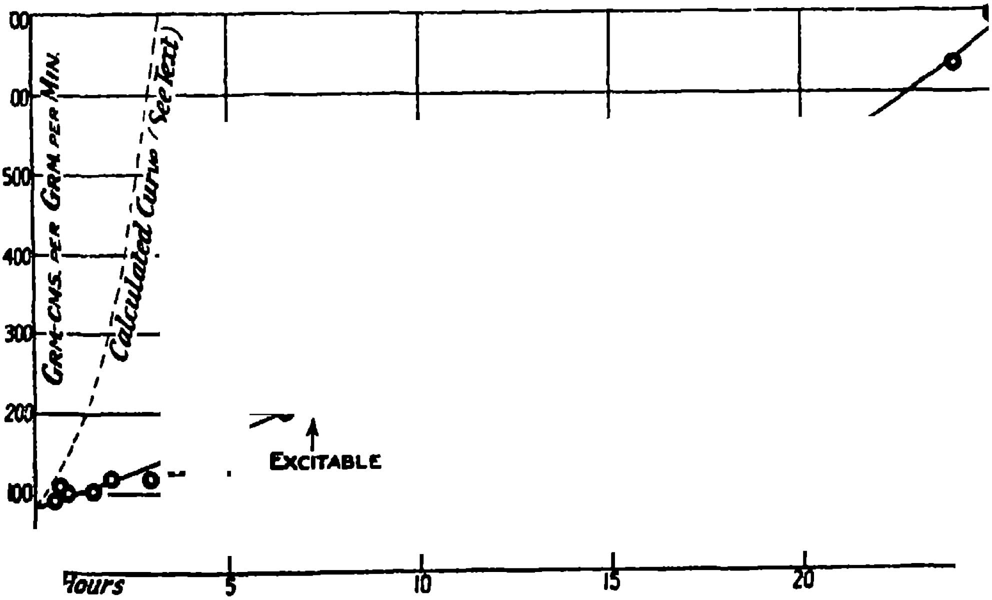

Fig. 7.—Increase of anaerobic resting heat-rate during prolonged survival without stimulation. Muscle in nitrogen at 19·2° C.

left at rest without oxygen exhibits a gradual increase in its heat-rate, which ultimately reaches high values.* The total heat in 25 hours amounted, in the above case, to 12·1 calories per gramme. The rate of increase is nearly uniform, not of the " compound interest " type. If the breakdowns represented by the increased heat-rate themselves caused a further increment in the heat-rate, the curve would be of a different kind, as shown by the broken line. In calculating the latter, the formula given above has been employed—

$$(\text{Increment in heat-rate}) = 0{\cdot}0113\ (\text{total heat liberated}),$$

the factor K = 0·0113 being calculated for 19·2° C.

Let y be the heat-rate and θ the time in minutes. Then, according to the above equation,

$$y = \text{a constant} + \mathrm{K}\int_0^\theta y\,d\theta,$$

or,

$$dy/d\theta = \mathrm{K}y,$$

from which

$$y = y_0 e^{\mathrm{K}\theta},$$

where y_0 is the initial value of y, and e is the base of the Napierian logarithms.

Expressing time in hours instead of minutes, and introducing the numerical value of K, this becomes

$$y = y_0\,(1{\cdot}97)^t.$$

Assuming y_0 to be 80, this equation gives the broken line on the left of fig. 7. It clearly bears no relation to the actual observations. The same thing is shown by numerous other experiments: whereas an increment in total heat *produced by stimulation* is followed invariably by a corresponding increment in the resting heat-rate, there is no correspondence between the slow increase in heat-rate at rest and the total heat produced by enhanced resting processes. It is clear that *the breakdowns underlying the increment in anaerobic resting heat-rate are quite different in their nature from those*—lactic acid formation, phosphagen changes, etc.—*occurring in muscular activity.*

The heat-rate during the first 10 hours of the experiment of fig. 7 increases at the uniform rate of about 19 per hour, or 0·32 per minute. If we suppose a uniform breakdown of glycogen, phosphagen, etc., and the resulting increase in oxygen-want, to be responsible for this uniform increase in heat-rate, according to the formula :—

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* It is of interest that in the experiment of fig. 7 the total anaerobic resting heat up to 7 hours was 1·4 calorie per gramme, at a stage when the muscle was still excitable. If this heat had come from lactic acid formation, the muscle would have been inexcitable long before.

(Increment in heat-rate) = 0·0113 (Heat produced by such breakdowns),

we may calculate that these breakdowns are accompanied by a heat-rate of 0·32/0·0113, *i.e.*, of 28 grm.-cms. per gramme per minute. This is less than the minimum anaerobic resting heat-rate described and discussed above ; it corresponds to only 0·0103 per cent. of lactic acid formed per hour. This would imply that the minimum resting heat-rate does not represent purely lactic acid formation, etc., but is still contaminated by a certain amount of heat liberated by other breakdown processes inevitably occurring in a dissected surviving muscle. This is possible. Among the values of the minimum resting heat-rate observed, some have been as low as 26 (13·2° C.), 37 (15·2° C.), 41 (18·7° C.), 44 (19° C.), 45 (19° C.). while others have been as high as 123 (20° C.) and 107 (18·6 C.). It is conceivable that injury due to removal from the animal may always lead, to some extent, to an increase in the minimum resting heat-rate, in some cases more than others, and that the true minimum rate, corresponding to lactic acid formation, etc., alone may at 20° C. be more in the neighbourhood of 30. If so, the rate of rise of the curve in fig. 7 would be fully in keeping with the data derived from stimulation.

8. *The Temperature Coefficient of the Resting Heat-Rate.*

A muscle was placed on the thermopile in pure nitrogen at 20° C. Its resting heat-rate was *57*. It was tetanised to complete exhaustion, and 43 minutes afterwards its heat-rate was *417*. The temperature of the thermostat and muscle chamber was then lowered to 0° C., at which it remained for 48 hours without observation, but with pure nitrogen steadily running through. At the end of that time the resting heat-rate of the dead muscle at 0° C. was *148*. It was then warmed to 20·75° C., and one hour afterwards the heat-rate was *1196*. Assuming the last two readings to correspond (an hour is the minimum time required to get a reliable reading after warming the system up), the temperature coefficient is 2·74 for 10° C.

A muscle was left in oxygenated Ringer at 0° C. all night (13 hours). After being " electrocuted " in pure nitrogen at 0° C. its heat-rate was *89*. Its temperature was raised to 19·5° C., and in 1¾ hours the heat-rate was *593*. Assuming the two readings to correspond, the temperature coefficient is 2·64.

From these experiments it is clear that the resting heat-rate of the inexcitable or " electrocuted " muscle has a temperature coefficient of the same order of size as other biochemical or vital processes.

One point of practical interest emerges from the above. It is clear that the resting heat-rate under anaerobic conditions, although largely reduced by cooling to 0° C., is still not negligible. A resting heat-rate of 148 represents 0·21 calorie per gramme per hour, or 5 calories per gramme per day. Continued for 10 days at this rate, 50 calories per gramme would be liberated, or about 5 per cent. of the total combustion energy of the tissue. The matter would seem to be of interest in connection with the "chilling" (as distinguished from the freezing) of imported meat.

9. *Is the Increment produced by Stimulation in the Anaerobic Resting Heat-Rate due to Increased Acidity of the Muscle?*

In discussing the cause of the increment in anaerobic resting heat-rate, one obvious possibility is that the increased acidity produced by stimulation may accelerate reactions previously going on at a low velocity. This possibility was tested by admitting pure carbon dioxide into the muscle chamber and seeing whether the deflection was increased thereby. According to a pair of observations made for me with the glass electrode by Mrs. P. Kerridge, of this Department, the *p*H of a frog's sartorius muscle placed in carbon dioxide falls from 7·0 to 6·0 approximately. The *p*H of a frog's muscle after stimulation to complete fatigue is stated by Meyerhof and Lohmann (7) to be about 6·2. Thus the introduction of pure CO_2 should lead to a change of hydrogen ion concentration about equal to that caused by complete fatigue.

When carbon dioxide is introduced into the chamber there is at first a large production of heat, lasting for about 45 minutes. When the carbon dioxide is washed out again with nitrogen there is an equal absorption of heat. These changes are to be attributed :—

(*a*) To the heat of solution of the gas ; and

(*b*) To the combination of the carbonic acid with the alkaline buffers of the muscle.

If the experiment be made carefully, introducing the gas slowly, the heat can be determined accurately from the area of the deflection-time curve. In four experiments made at an average temperature of 20·4° C., the following values were found on unstimulated or recovered muscles, previously in nitrogen :—

0·342, 0·406, 0·359, 0·430 calorie per gramme.
Mean, 0·384.

In fatigued muscles the heat liberated by carbon dioxide is considerably less, owing to the fact that much of the alkali has already been used in neutralising lactic acid : for example—

(1)	Heat produced by stimulation	0·672	calorie per gramme.
	Heat by subsequent CO_2	0·284	,, ,, ,,
(2)	Heat produced by complete fatigue	0·893	,, ,, ,,
	Heat by subsequent CO_2	0·206	,, ,, ,,

Of these quantities the part due to simple solution of carbon dioxide can be calculated. The solubility of carbon dioxide in water at 20·4° C. is 0·868 : in muscle we will assume it to be 86 per cent. of this, namely, 0·75. The heat of solution of carbon dioxide at 20° C. is 4810 cals. per gramme mol. (8), while the heat absorbed in the removal of nitrogen is small enough to be neglected. A muscle immersed in pure carbon dioxide takes up, in physical solution, 0·75 c.c., which leads to a liberation of 0·138 calorie. Thus, in the resting muscle, subtracting this quantity from the mean value 0·384 cal., we find 0·246 cal. as due to the heat of combination of carbonic acid with muscle alkalies. If muscle be similar to blood, 1 gramme molecule of carbon dioxide neutralised by alkali-proteins will liberate 12,000 calories (8). Thus the carbon dioxide taken up and combined in one gramme of muscle will be $\frac{0 \cdot 246 \times 22,400}{12,000}$, *i.e.*, 0·46 c.c. In the completely fatigued muscle it can similarly be calculated that the carbon dioxide taken up in combination is 0·13 c.c. per gramme, while in the partially fatigued it is 0·27 c.c. per gramme. These values are only rough, but they allow us to make an approximate calculation of the hydrogen-ion concentration.

The dissociation constant of carbonic acid is 3×10^{-7}—that is to say, the hydrogen-ion concentration can be calculated from the equation

$$cH = 3 \times 10^{-7} \frac{(\text{dissolved } CO_2)}{(\text{combined } CO_2)}.$$

In the resting muscle treated with carbon dioxide, introducing 0·75 as the "dissolved CO_2" in the numerator, and 0·46 as the "combined CO_2" in the denominator, we find the cH to be $4 \cdot 9 \times 10^{-7}$, the pH to be 6·31 : in the partly fatigued muscle, similarly treated, the calculated pH is 6·08 : in the completely fatigued muscle, 5·76. It is clear that immersion in carbon dioxide makes a considerable alteration in the hydrogen-ion concentration of a muscle.

The treatment of muscles with pure carbon dioxide causes, in general, no increase in the resting heat-rate.

No. of Experiment.	No of shocks in N_2.	Heat-rate in N_2	Heat-rate in CO_2.
1	60	45 scale divisions	24 scale divisions.
2	60	35 ,, ,,	34 ,, ,,
3	120	64 ,, ,,	55 ,, ,,
4	60	33 ,, ,,	20 ,, ,,
5	0	146 grm.-cms. per grm. per min.	136 grm.-cms. per grm. per min.
6	0	63 ,, ,,	63 ,, ,,
7	0	85 ,, ,,	154 ,, ,,
8	0	114 ,, ,,	155 ,, ,,
9	Fatigue	482 ,, ,,	508 ,, ,,
10	Complete fatigue	821 ,, ,,	633 ,, ,,

There would seem rather to be a tendency, on the whole, for treatment with carbon dioxide to decrease the resting heat. Certainly in the resting, or in the moderately stimulated muscle (1, 2, 4, 5, 6, 7, 8), there is no sign of an increment in heat-rate equal to that occurring in a muscle stimulated to exhaustion, as there should be if the hydrogen-ion concentration were the deciding cause. We may conclude, therefore, that *the increased acidity provoked by stimulation is not the cause of the increment observed in resting heat-rate.*

10. *The Reversibility in Oxygen of the Effect of Anaerobic Stimulation in Increasing the Resting Heat-Rate.*

A muscle at 13·2° C. in pure nitrogen exhibited a resting heat-rate of *26*. Stimulated by 183 break-shocks, it gave the response shown in fig. 1 and heat 15,200, after which its resting heat-rate in nitrogen was *145* (see fig. 2). It was then allowed to recover for 2½ hours in oxygen, after which its resting heat-rate in oxygen was *94*. It was then stimulated in oxygen by 90 break-shocks, giving the second response of fig. 1, and after recovery its resting heat-rate in oxygen was *115*. A third series of 30 shocks in oxygen gave the third response of fig. 1, and after recovery in oxygen its resting heat-rate was *100*. It is clear from fig. 1 that the muscle was in no way irreversibly injured by its anaerobic stimulation, although (calculated from the heat) nearly 0·1 per cent. of lactic acid was liberated, and we see that the recovered muscle exhibited a considerably smaller heat-rate in oxygen than previously (after stimulation) in nitrogen. It is almost inconceivable that the same chemical reaction could give out less heat in oxygen than in nitrogen: *therefore recovery in oxygen must have inhibited a reaction previously occurring.*

Many other experiments have been performed illustrating the same phenomenon. A muscle after 43 minutes in oxygen at 20·7° C. showed a resting heat-rate in oxygen of *250*: after a series of 77 shocks (see fig. 8, lower record), and 32 minutes recovery, the resting heat-rate in oxygen was *242*.

Fig. 8.—Experiment of 22.3.28. Bottom, Series I, 77 maximal break-shocks in oxygen, isometric record. Top, after recovery, Series II, 78 shocks in nitrogen. Read from left to right.

The oxygen was then replaced by pure nitrogen, and in 25 minutes the heat-rate was *61*. A series of 78 shocks (see fig. 8, upper record) then gave heat 9,040, after which the resting heat-rate in nitrogen was *168*. Oxygen was then admitted, and in 45 minutes recovery heat 9,460 was liberated, and the resting heat-rate reached a value of *268*. Finally the oxygen was replaced by nitrogen, and in 30 minutes the heat-rate had fallen to *64*. From this we see that after complete recovery in oxygen ((recovery heat) ÷ (initial heat) = 1·05) the resting heat-rate in nitrogen returned to the value which obtained before anaerobic stimulation. *The change in the resting heat-rate produced by anaerobic stimulation was fully reversed by recovery in oxygen.*

A third experiment may be quoted, showing a partial and not a complete reversal. A muscle after 102 minutes in oxygen had a heat-rate in oxygen of *145*. The oxygen was replaced by nitrogen and in 36 minutes the heat-rate in nitrogen had become constant at *111*. A series of 191 shocks in nitrogen led to a liberation of heat 23,100 (≡ 0·14 per cent. lactic acid), and to a final heat-rate in nitrogen of *458*. Oxygen was admitted, and recovery proceeded for 67 minutes, leading to a heat liberation of 7,270 ; this is only 31 per cent. of the initial stimulation heat : recovery was not complete, as was shown also by the subsequent behaviour. At the end of 67 minutes in oxygen the heat-rate in oxygen was constant at *325*, more than the previous value in oxygen but less than that after stimulation in nitrogen. Two shocks now showed a largely diminished mechanical response, with but little reduction in the heat-production. The oxygen was finally replaced by nitrogen, and in half an hour the heat-rate in nitrogen had fallen to *218*. This is more than the initial heat-rate in nitrogen, but considerably less than that after stimulation. The muscle was now nearly inexcitable. Here we have an example of *partial oxidative recovery causing a considerable but incomplete reversal of the effect of anaerobic stimulation.*

A fourth experiment illustrates the same thing.

	Resting heat-rate.
(1) Muscle at 20·5° C., 65 minutes in oxygen	190
(2) Oxygen replaced by nitrogen, in 32 minutes	57
(3) 76 shocks, heat, 11,000, in nitrogen	219
(4) 41 minutes' recovery in oxygen, heat 11,480	288
(5) Oxygen replaced by nitrogen, in 30 minutes	146
(6) " Electrocuted "	475

After recovery in oxygen (line 4), the mechanical response to a shock was only 35 per cent. of its original value: correspondingly we find, by comparing lines (2), (3) and (5), that the anaerobic resting heat-rate has been diminished by oxidative recovery, but not to its low initial level. "Electrocution" finally produced its characteristic high value.

We may conclude that the effect of anaerobic stimulation in increasing the resting heat-rate *may* be completely reversed by recovery in oxygen, with a return of the muscle to its original condition. The reversal, however, is often only partial: too considerable an anaerobic breakdown induced by stimulation may lead to an irreversible increase in the heat-rate, and in such cases the muscle tends to fail. The heat-rate in oxygen, after recovery, may actually be less than in previous nitrogen after stimulation, showing that the oxygen has inhibited some breakdown occurring in the muscle.

No exact quantitative data can be produced, but the writer has a strong personal impression that those muscles which exhibit a high anaerobic resting heat-rate are failing, or liable to fail, while those which show a low heat-rate are in good condition and likely to stand stimulation well. The causes of failure are unknown, but a connection between liability to failure and those changes which lead to a high anaerobic heat-rate would seem to be important in any discussion of the maintenance of the normal life of the organism.

Discussion.

Oxidation is necessary in order to maintain the normal life, structure and function in an animal cell. Some cells—*e.g.*, those of muscle—may continue to function for a period without oxygen, by employing the "accumulator-mechanism" afforded by the power of forming lactic acid from glycogen; others—*e.g.*, those of the kidney—cease their normal function as soon as oxidation is inhibited by cyanide (Starling and Verney, (9)). A third type has recently been found in vertebrate nerve (10), (11), (12), (2), (13), in which the functional capacity to transmit an impulse does not cease as soon as oxygen is withdrawn, yet apparently activity is always followed by a recovery process which can scarcely be other than oxidative in nature, but derives its energy from a source other than the use of molecular oxygen. When finally the function of the nerve disappears under oxygen lack, it can be brought back in part at once by the re-admission of oxygen, but it requires an hour or more for a complete restoration to its original state; whereas a full supply of oxygen would be available within a minute or two. Lack of oxygen, long enough continued, ends in the death of the nerve.

The investigations of Warburg (3) have shown that malignant tumour-tissue is characterised by an excessive employment of energy derived from the "fermentative" lactic acid-forming reaction, and a diminished use of oxidative breakdown. The malignancy may perhaps be associated with, and measured by, the diminished power of using oxygen in the reactions required to maintain the life and normal micro-physical architecture of the cell. If the cell is to continue to function at all it must employ energy, which it derives—since oxidation is insufficient—from anaerobic breakdown. The lack of oxidative power, and not the excessive "fermentative" capacity, may be the essential factor.

We are led by such considerations to the very fundamental question: What is the rôle of oxidation in the living but completely resting tissue ? Why is it necessary for the isolated nerve, if it is to continue *to be able* to transmit an impulse, to persist in using oxygen at the rate of 0·00035 c.c. per gramme per minute ? The nerve is completely at rest, no detectable change is taking place in it, yet about 10,000 molecules of oxygen are being consumed every minute in every cubic μ. Some clue to the solution of the problem may be given by the experiments described above. If a tissue be broken up by mechanical or chemical means, reactions rapidly set in which previously were impossible, catalysts are brought in contact with materials from which they were previously held apart, and the cell rapidly degenerates from an organised system which was previously in dynamic equilibrium to a complex medley of biochemical processes and substances.

It is clear from the preceding pages that although the use of molecular oxygen may be completely prevented, large amounts of energy are nevertheless available in muscle for anaerobic breakdown, amounts far in excess of that which can be derived from any possible formation of lactic acid. The reactions underlying this liberation of energy are unable to proceed so long as the muscle can maintain its normal basal level of oxygen consumption: cut off the molecular oxygen, however, and they commence increasing in intensity in direct linear proportion to the degree of oxygen want, the "oxygen debt," of the tissue. If oxygen be re-admitted before the degenerative processes have gone too far, these breakdowns are inhibited, substances previously able to react with one another are held apart again, and the muscle returns to its normal resting state. Allow the degenerative changes produced by lack of oxygen to proceed too far, and no recovery is possible, the agencies capable of holding things apart so that they cannot react have been destroyed, and the organised system rapidly becomes a chaos of biochemical processes.

In ascribing a more definite and material existence to those agencies which, in the presence of oxygen, inhibit reactions which otherwise, sooner or later, proceed to the destruction of the cell, the great increase in anaerobic heat-rate induced by " electrocution " is significant. Stimulation to extreme fatigue causes a large increase : " electrocution " causes a larger one. The " electrocution " even of a completely inexcitable muscle may lead to a considerable increment in the resting heat-rate. It is difficult to see how a strong alternating current could provoke chemical reactions in a homogeneous medium : it would seem more likely that powerful electrical potential differences applied to a tissue may actually puncture or shatter the membranes, or interfacial layers, which normally maintain the heterogeneity of the cell. Thus we should tend to look for the inhibitory agencies which uphold the dynamic equilibria of life, and prevent unwanted reactions from occurring, not in any mysterious chemical control exerted in a homogeneous medium, but in actual material membranes and interfacial layers.

The degree of oxygen-want of a tissue, its " oxygen debt," is determined by the quantity of energy liberated by previous anaerobic stimulation, by the amount of lactic acid set free. Acid, as such, has been shown by the experiments with carbon dioxide not to provoke an increment in the resting heat-rate. It is equally unlikely that any associated changes in the phosphate compounds of the muscle could produce the effect. In view of the power possessed by oxygen of inhibiting or reversing the increment in resting breakdown, it is natural *to regard oxygen-want, as such, as the agency provoking degenerative change*. Why, or how, the rate at which these degenerative breakdowns occur in an anaerobic muscle, can be a linear function of the degree of oxygen-want, it is not easy to say. It may be, as in nerve, that there is a source of oxygen in other than molecular form available for the life of the tissue, and that the essential interfaces guarding the integrity of the cell are progressively deprived of this source as the oxygen-want of the tissue increases. With a high lactic acid concentration, substances may be liable to chemical reduction which were stable with a low one.

Further researches on the oxidation-reduction potentials of living systems may throw light on the matter. For the moment one can only record the phenomena, which seem to present a fairly exact form under the conditions named, and speculate somewhat vaguely about them. Whether they will appear at all, or so rapidly, under other conditions, *e.g.*, in muscles not dissected from their limbs, cannot be predicted. They do occur under certain conditions, and their occurrence requires an explanation. Renewed chemical studies of muscles and other tissues, kept under strictly anaerobic conditions, are suggested. The nature of reactions which can liberate, without oxidation, such relatively large amounts of energy would seem particularly worthy of investigation.

Summary.

1. The resting heat-rate of frogs' sartorius muscles has been investigated, in oxygen and under strictly anaerobic conditions.

2. (*a*) The resting heat-rate in oxygen agrees with existing determinations of the rate of oxygen consumption.

(*b*) Survival in oxygen, and stimulation followed by recovery in oxygen, tend to diminish rather than to increase the resting heat-rate.

3. The minimum resting heat-rate in the absence of oxygen is attained within half an hour in nitrogen, and is sufficiently accounted for by lactic acid production.

4. (*a*) Stimulation produces an immediate increase in the anaerobic resting heat-rate, which under conditions of advanced fatigue may reach considerable values.

(*b*) This increase persists for many hours, long after the muscle has become inexcitable.

(*c*) A linear relation exists between the increment in anaerobic resting heat-rate and the energy liberated by previous anaerobic stimulation.

(*d*) A critical examination shows that the phenomena described are not due to any technical error, but to properties of the muscle tissue itself.

(5) "Electrocution," *i.e.*, excessive electrical stimulation, produces a great increase in the anaerobic resting heat-rate.

(6) The absolute values are discussed. The total heat in 8 hours due to the anaerobic resting heat-rate may be as great as 5 calories per gramme of muscle: the maximum rate may be as high as 1·4 calories per gramme per hour. The quantities involved are so large that the phenomenon described cannot be due simply, or mainly, to enhanced lactic acid formation: it must be attributed to other anaerobic breakdowns involving far more energy than this.

(7) Resting anaerobic survival without stimulation leads gradually to the same high heat-rate.

(8) The increased resting heat-rate provoked by anaerobic stimulation, or survival, has a temperature coefficient of 2·7 for 10° C.

(9) (*a*) Increased acidity is not the cause of the phenomenon. Immersing

a muscle in pure carbon dioxide tends rather to decrease than to increase the resting heat-rate.

(*b*) The immediate effect of introducing carbon dioxide is a large production of heat, caused by its solution and combination in the muscle. This production of heat is diminished by previous stimulation. A corresponding absorption of heat occurs when the carbon dioxide is removed.

(10) The effect of anaerobic stimulation in increasing the resting heat-rate can be reversed, partially or completely, by recovery in oxygen. The possibility of oxidation leads to the inhibition of reactions previously occurring.

(11) The rôle of oxidation in maintaining the chemical and physical architecture of the cell is discussed. The degenerative changes set up by anaerobic stimulation, or survival, proceed at a rate determined at any moment by the degree of oxygen-want. Oxidation at rest is concerned with upholding the integrity of boundaries or membranes, which are essential if the organised system is not to become a chaos of biochemical processes.

The expenses of this research have been borne by a grant from the Foulerton Fund of the Royal Society.

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The Absolute Value of the Isometric Heat Coefficient Tl/H *in a Muscle Twitch, and the Effect of Stimulation and Fatigue.*

By A. V. Hill, F.R.S.

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It is not technically possible to determine directly the lactic acid set free in a single muscle twitch. It is necessary to calculate it from the initial heat-production, or from the tension developed. The anaerobic liberation of 1 gramme of lactic acid in muscle is accompanied, according to Meyerhof, by the production of 385 calories of heat (1). This leads to the equation :—

$$1 \text{ gramme-cm. (heat)} \equiv 6\cdot14 \times 10^{-8} \text{ gramme lactic acid.} \qquad \text{(I)}$$

The isometric coefficient of lactic acid, defined for a twitch or a series of twitches by the equation*

$$K_m = \frac{\text{(grammes tension developed) (cms. muscle length)}}{\text{(grammes lactic acid produced)}},$$

has been the subject of much investigation by Meyerhof and his colleagues (2, 3, 4, 5). Matsuoka, for the frog's sartorius muscle in Ringer's solution, found a mean value of $1\cdot05 \times 10^8$ (variation $0\cdot69$ to $1\cdot36$). Meyerhof and Lohmann, for the frog's gastrocnemius, gave $1\cdot40 \times 10^8$ as a mean, while Meyerhof and Schulz gave $1\cdot43 \times 10^8$ (variation $1\cdot12$ to $1\cdot66$). In the gastrocnemius, however, the fibres are not straight, and do not run parallel to the muscle length ; consequently it is necessary to multiply (see Mashino (6), A. V. Hill (7)) the value so found by a factor of roughly $0\cdot63$ to allow for the skew disposition of the fibres. This gives, when corrected, $0\cdot9 \times 10^8$ for the gastrocnemius, so that taking account of the value $1\cdot05 \times 10^8$ found by Matsuoka for the sartorius, the round figure 1×10^8 may be accepted. This leads to the equation :—

$$1 \text{ gramme-cm. (tension-length)} \equiv 10^{-8} \text{ gramme lactic acid.} \qquad \text{(II)}$$

From equations (I) and (II) we may calculate at once the lactic acid produced in a twitch, given either (I) H, the heat produced, or (II) T, the tension developed. Combining them we find a mean value for Tl/H, viz.,

$$\frac{\text{Tl}}{\text{H}} = 6\cdot14. \qquad \text{(III)}$$

---

* Meyerhof defines it in terms of kilogrammes of tension developed and milligrammes of lactic acid produced.

It is assumed that H, the heat, is the "initial heat." It will be shown in a subsequent paper that in a series of twitches there is little, or no, delayed anaerobic heat.

It is of interest to compare the mean value of Tl/H so calculated with one deduced directly from simultaneous observations of T and H. By means of the methods described in the two preceding papers, such observations have been made more accurately than in previous investigations, (*a*) on Tl/H in single twitches in the usual way, and (*b*) on ΣTl/H in series of twitches, where ΣTl is the sum of the tensions developed multiplied by muscle length, and H is total heat calculated from the observed area of the galvanometer deflection-time curve. In every case the muscle was in pure nitrogen, sometimes treated with cyanide, after having been immersed for some time previously in neutral phosphate-Ringer (usually 7 to 10 mgms. per cent. P). The muscle had not been stimulated previously, except for a few twitches.

Twelve values for Tl/H (each the mean of several observations) in twelve different experiments are as follows: 4·34, 4·58, 5·37, 6·03, 6·25, 6·30, 6·40, 6·70, 6·97, 7·40, 7·65, 8·30; mean value, 6·36.

Twelve values for ΣTl/H in twelve different experiments are as follows, the number of twitches in each series being given in brackets after the value of ΣTl/H: 4·60 (63), 4·75 (65), 4·86 (31), 5·12 (60), 5·25 (32), 5·47 (183), 5·74 (32), 6·10 (77), 6·72 (78), 6·98 (50), 7·24 (63), 7·30 (62), 7·48 (191); mean value, 5·97.

The average, therefore, of the two mean values, viz., 6·16, is in close agreement with that deduced above from Meyerhof's lactic acid data, and the variation from one muscle to another is no greater than that observed in the lactic acid experiments. In the case, at any rate, of the myothermic observations, the variation from one experiment to another is not due to experimental error—the error cannot be more than 3 or 4 per cent. and the average must be much less—it should be attributed to the unavoidable differences existing between different muscles similarly treated and apparently in good condition.

The assumption has been made in the above comparison that stimulation to partial fatigue (as is necessary for lactic acid measurements) does not affect the isometric coefficient for lactic acid, so that a series of 100 to 200 twitches should give the same result as a group of three or four. Meyerhof (2) showed that in a muscle pushed to extreme fatigue the isometric coefficient falls considerably: two experiments may be quoted:—

(1) First 145 twitches, $K_m = 0 \cdot 77 \times 10^8$ (after applying the correction factor of 0·63 for the gastrocnemius).

Succeeding 350 twitches, $K_m = 0 \cdot 52 \times 10^8$.

(2) First 210 twitches, $K_m = 0 \cdot 77 \times 10^8$.

Succeeding 590 twitches, $K_m = 0 \cdot 52 \times 10^8$.

This effect can be confirmed by the myothermic method: there is no doubt that in the last stages of exhaustion the ratio Tl/H falls considerably. Over a more moderate range, however, in a muscle in good condition, it remains remarkably constant, as is shown in Tables I and II.

In Table I the degree of previous anaerobic activity is given by the amount of heat liberated (calories per gramme): complete fatigue is usually reached when about 1 cal. per gm. (*i.e.*, about 0·26 per cent. of lactic acid) has been produced. In Table II the heat set free in each series is given, so that the degree of previous activity can be inferred by adding up the previous heats. In Table I are given the results for Tl/H in single twitches, obtained as usual, in every case but one, in absolute units. In Table II are given results for ΣTl/H for series of twitches, ΣTl being obtained by simple addition, H from the area of the deflection-time curve of the galvanometer, as described in the preceding papers: ΣTl/H is expressed in absolute units.

Table I.—Effect of Previous Stimulation on the Value of Tl/H in a Single Anaerobic Twitch.

Experiment I of 27.10.27.—Pure N_2 after Ringer, 20 mgms. per cent. P, *p*H 7·6. Tetani interpolated between groups of three test-shocks.

Duration interpolated tetanus: seconds . ..		0·45		0·45		0·45		0·90		1·8		1·8		1·8		1·8		1·8		1·8	
Total cals. per gm. in previous stimulation	0·009		0·064		0·117		0·169		0·242		0·350		0·447		0·530		0·600		0·654		0·700
Tl/H, mean of 3 twitches	3·54		3·62		3·51		3·51		3·42		3·21		3·34		3·47		3·70		3·86		4·05

Experiment II of 26.10.27.—Pure N_2 after Ringer, 10 mgms. per cent. P, *p*H 7·4. Groups of three test-shocks and 1-second tetani alternating.

Previous cals. per gm.	0·018	0·124	0·194	0·258	0·315	0·366	0·410	0·450	0·481	0·510	0·535	0·555	0·590
Tl/H	4·20	4·30	4·07	4·10	4·27	4·36	4·28	4·12	4·04	4·00	3·84	3·66	3·60

Experiment I of 26.10.27.—As Experiment II of 26.10.27 above. No calibration. T/H in arbitrary units. Groups of four test-shocks and single 4-second tetani alternating.

T/H arbitrary units	7·8	8·9	9·1	9·3	9·1	8·5	7·8	7·4

Experiment II of 27.10.27.—Pure N_2 after Ringer, 20 mgms. per cent. P, *p*H 7·4. Groups of three test-shocks and single 2-second tetani alternating.

Previous cals. per gm.	0·011	0·132	0·237	0·323	0·392	0·446	0·490
Tl/H	3·38	3·74	3·74	3·80	3·80	4·11	4·04

Table II.—Effect of Previous Stimulation on the Value of $\Sigma Tl/H$ in a Series of Anaerobic Twitches.

Experiment of 22.12.27.—Total heat in stimulation 0·53 cal. per gm.

Number of twitches	65	63	63	64
Heat produced : cals. per gm.	0·15	0·14	0·13	0·11
$\Sigma Tl/H$	4·75	4·51	4·32	4·81

Experiment of 14.10.27 *and* 15.10.27.—4 hours in Ringer + 20 mgms. per cent. P. Then 17 hours in O_2, then in N_2. Total heat in stimulation 0·82 cal. per gm.

Number of twitches	37	38	79	159	122
Heat produced : cals. per gm.	0·063	0·075	0·177	0·334	0·168
$\Sigma Tl/H$	3·92	4·23	4·05	4·11	3·74

Muscle then left in oxygen : after 10 hours contracted strongly to stimulation.

Experiment of 3.1.28.—In N_2–HCN. Total heat in stimulation 0·610 cal. per gm.

Number of shocks	31	31	64	62	188
Heat produced ; cals. per gm.	0·086	0·081	0·137	0·121	0·185
$\Sigma Tl/H$	4·86	4·56	4·52	4·72	4·56

Experiment of 25.1.28.—In N_2–HCN. Total heat in stimulation 0·47 cal. per gm.

Number of shocks	32	32	32	64
Heat produced : cals. per gm.	0·107	0·106	0·103	0·155
$\Sigma Tl/H$	5 74	5 08	4·43	3·14

Experiment of 4.1.28.—In N_2–HCN. Total heat in stimulation 0·64 cal. per gm.

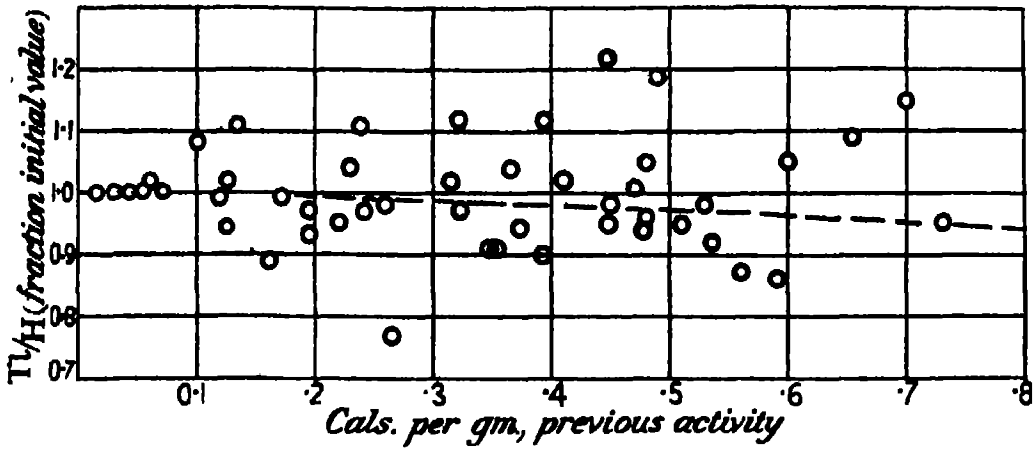
Number of shocks	63	310 to extreme fatigue.
Heat produced : cals. per gm	0·108	0·53
$\Sigma Tl/H$	7·24	6·82

Except where stated, the muscle had been treated with neutralised phosphate-Ringer, 7 to 10 mgms. per cent. P. Except in the last experiment quoted, stimulation was not to extreme fatigue. The heat produced in each separate series is given.

One comment should be made on the data in Tables I and II. It is quite easy to get a contrary result, viz., a large *fall* in Tl/H as fatigue sets in, by employing muscles which are rapidly deteriorating. In all the above cases the muscles behaved well, and gave a long series of responses without undue depreciation : the experiments quoted were chosen *simply* because the muscles were, judging from their mechanical response, in good condition up to the end of stimulation. In every case the resting heat-rate in nitrogen at the beginning (see the preceding paper) was low, which is a further sign of good condition.

The values given in Tables I and II have been shown graphically in the accompanying figure by the following treatment : The first entry for Tl/H in each experiment was taken as unity, and subsequent values were expressed as a fraction of the initial one. Each fraction was then plotted as a function of the amount of heat liberated in previous stimulation. The case of Table I needs no comment. In Table II the total heat liberated *up to the middle* of the series under consideration is taken as the " previous " heat.

The method of plotting exaggerates the " scatter " for two reasons : (*a*) any error or abnormality in the first reading of an experiment is perpetuated throughout the experiment by dividing the other readings by it, and (*b*) the diagram is cut off at the value 0·7 instead of being continued down to zero. It is obvious, nevertheless, that there is very little, if any, change in Tl/H as the result of previous activity: the line Tl/H = 1 runs practically through the middle of the points. There is apparently a slight tendency for the ratio to fall, and the broken line is so drawn as to have the same number of points above as below it. To half fatigue (say 0·5 cal. per gm.) there is only about 3 per cent. diminution in Tl/H, if we accept the broken line; even at 80 per cent. fatigue (say 0·8 cal. per gm.) there is only about 6 per cent. diminution. Practically speaking, therefore, we may say that previous activity has no effect on the ratio Tl/H, except during the extreme stages of fatigue.



Graphic representation of results in Tables I and II. The first entry for Tl/H in each experiment was taken as unity; all subsequent entries were divided by the first, and then plotted as a function of the heat liberated in previous anaerobic activity. In the case of Table II, previous activity was reckoned to the middle of the series considered.

We may conclude that in a single twitch the processes leading to the development of tension and heat are the same as in a long series of twitches: thus, in order to determine the lactic acid produced in a single average twitch we may employ equations (I) and (II) above. Any error involved in so doing will be almost entirely that due to the random variation of the tissues employed, and will not be affected by the fact that the constants of the equations were derived from long series of twitches.

A certain special importance attaches to these results at the present moment, owing to recent work on the chemistry of "phosphagen." Phosphagen is an unstable compound of creatine and phosphate—probably combined with other substances—which has been found to break down during the activity of muscle, and to be rapidly reformed afterwards in the presence of oxygen. (See Eggleton and Eggleton (8), (9), (10).) Recent work from Prof. Meyerhof's laboratory in Berlin has shown certain unexpected phenomena, which lead one to suspect that the phosphagen is not really broken down at all, but in some sense "unstabilised" by stimulation. This work will be published shortly in a paper by D. Nachmansohn, the MS. of which Prof. Meyerhof was kind enough to send to Mr. Eggleton. The essential points are as follows :—

At rest in a fresh muscle, 73 per cent., approximately, of the "inorganic" phosphate, as usually determined, really consists of phosphagen. The "isometric coefficient" for phosphagen,

$$K_p = \frac{\text{(grammes tension) (cm. muscle length)}}{\text{(grammes inorganic } H_3PO_4 \text{ produced)}},$$

is not constant, or approximately constant, as is the "isometric coefficient" for lactic acid K_m defined above, but increases rapidly as the degree of previous activity increases. For example, in 42 twitches it was $0 \cdot 56 \times 10^8$, in 85 twitches $0 \cdot 78 \times 10^8$, in 325 twitches $2 \cdot 4 \times 10^8$ (all corrected by the reducing factor 0·63 for the gastrocnemius). Whereas in the short series 1·5 times as many phosphagen molecules "break down" as lactic acid molecules are formed, in the later stages of the long series the ratio, instead of being 1·5, is only about 0·1. The same effect is shown in a tetanic stimulus: in a single 5-second tetanus, or in two, *twice* as much phosphagen may be broken down as lactic acid produced, while in a series of 6 or 12 the ratio may be reversed: indeed, in the later stimuli, practically no phosphagen appears to break down at all.

Now it has been shown by Meyerhof and Suranyi (1) that the breakdown of phosphagen *in vitro* leads to the liberation of 150 calories per gramme of H_3PO_4 set free. If the major portion of the phosphagen present in muscle really broke down in the early stages of a long series of twitches, one could scarcely fail to detect the fact from the gradual rise in Tl/H that would occur as the rate of phosphagen breakdown fell off. Such a rise definitely does not occur. There is no doubt that the isometric coefficient for lactic acid remains constant up to a fair degree of activity, while it has been shown above that the isometric heat coefficient is practically unchanged up to a high degree of activity. These facts seem to preclude the possibility that the heat observed is largely made up, in the early stages of activity, by a breakdown which is nearly as important (from the thermal aspect) as the reaction producing lactic acid, but during the later stages assumes a negligible importance. If the breakdown of phosphagen in the living muscle really liberated 150 calories per gramme of H_3PO_4 set free, we could not fail to detect its presence from the *rise* of Tl/H with advancing fatigue. It seems necessary to assume either that purified phosphagen has very different thermal properties from the substance existing in the living muscle, or that the breakdown does not really occur; perhaps the "breakdown" should be regarded rather as an "unstabilisation" of some kind, allowing the phosphagen to be broken down by the chemical treatment necessary for its estimation, to which it is normally resistant. This suggestion is, in fact, made by Nachmansohn in his paper, on other grounds.

Summary.

1. Meyerhof's experimental results on the relations between heat, tension and lactic acid in a series of isometric muscle twitches may be expressed by the following equations for the case of a muscle with fibres parallel to its length :—

1 gramme-centimetre (initial heat) $\equiv 6\cdot14 \times 10^{-8}$ gramme lactic acid.

1 gramme-centimetre (tension-length) $\equiv 10^{-8}$ gramme lactic acid.

$$Tl/H = 6\cdot14.$$

2. Direct observations of T and H by the improved methods described in the previous papers give a mean value of the isometric heat coefficient, $Tl/H = 6\cdot16$. The rather wide variations observed experimentally in this coefficient are not due to errors of observation but to differences occurring between the muscles employed, in spite of all precautions to ensure uniformity.

3. Previous anaerobic activity, liberating a large fraction of the whole energy available but not pushed to extreme fatigue, has little or no effect on the isometric heat coefficient Tl/H. The effect, if any, is in the direction of a slight reduction. In extreme fatigue, however, the reduction is large.

4. The equations, therefore, in (1) above, which were deduced from observations on a large number of twitches, are applicable without change to the case of a single twitch.

5. Recent work on "phosphagen"—a labile phosphate-creatine compound which appears to break down in activity and to be re-formed in oxidative recovery—has shown (*a*) that *in vitro* the breakdown of purified phosphagen leads to the liberation of about 150 calories per gramme of H_3PO_4 set free, and (*b*) that *in vivo* the "breakdown" occurs preponderatingly in the early stages of a long series of twitches. If the phosphagen "breakdown" *in vivo*

really liberated the heat found for the breakdown of the purified material *in vitro*, it could not fail to cause an obvious *increase* in the isometric heat coefficient as activity progressed and fatigue set in. Such an increase does not occur; either, therefore, purified phosphagen is to be regarded as a very different substance (from the thermal aspect) from that existing in the living muscle, or the phosphagen "breakdown" *in vivo* is to be regarded rather as an "unstabilisation," causing the material to break down under chemical treatment to which it is normally resistant.

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The Absence of Delayed Anaerobic Heat in a Series of Muscle Twitches.

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In 1922, Hartree and Hill (1) described a delayed heat-production after a short tetanic stimulus in a muscle deprived of oxygen, amounting to about one-third of the recovery heat in oxygen. In 1923 the same authors (2) re-examined the matter, with greater precautions to exclude oxygen, and found a " most probable " value for this delayed anaerobic heat of about one-quarter of the initial, or one-sixth of the recovery, heat. The existence of this heat has remained an obscure phenomenon, a complication in an otherwise comparatively simple scheme, and a further attempt was made by Furusawa and Hartree (3) in 1926 to trace its source. In spite of all precautions to exclude oxygen, and to obviate physical effects (lack of uniformity in the muscle, etc.) which might produce the same apparent result, the delayed heat persisted, and Furusawa and Hartree concluded that its minimum value was about 12 per cent. of the initial heat; in many cases it was more, sometimes much more. It should be noted that all the investigations referred to dealt with a short tetanus, not with a single twitch.

The increment produced by stimulation in the resting heat-rate of a muscle under anaerobic conditions, described in a previous paper of the present series, is one of the factors responsible for the effect discussed. Unless the galvanometer-zero, and the temperature of the thermopile-chamber, be extremely constant, it is easy to misinterpret the permanent shift of position of the galvanometer after stimulation in nitrogen, and to deduce the existence of a long-continued slow production of heat gradually diminishing to zero. The reactions underlying the increment in the resting heat-rate are not part of the process of activity itself, although induced by it, and it is incorrect to attribute the energy they liberate to the preceding contraction; this is obviously the case, since the increment in heat-rate we know now to be permanent, so that the energy liberated is not constant but proportional to the time during which one chooses to follow the galvanometer deflection. It will be shown, indeed, by Hartree and Hill in a later paper of this series that such anaerobic delayed heat as really exists, in the case of a tetanus, occupies only a minute or two after the stimulus, the long continued part of the delayed heat, described in each of the three papers cited above, is an error due to a misinterpretation of the facts, which could not then be observed with the same accuracy as is possible now

The case of a single twitch has never been examined in this respect The reason is that the amount of heat liberated is so relatively small, when compared with that in a tetanus, that experimental error would tend to mask the effect looked for What has proved so difficult of decision, in the case of a tetanus with its relatively large amount of heat, would clearly prove even more difficult in the case of a single twitch It is obviously important, however, if possible, to investigate this other type of contraction for (1) it represents the fundamental unit of muscular activity on which all other types are based, and (2) much recent work has been done on the chemical changes in muscle, by Meyerhof on the one hand and by Embden on the other, in which single twitches have been employed By measuring the heat, not of a single twitch but of a succession at equal intervals as is now possible, the experimental difficulty has been resolved, and it will be shown below that in a series of twitches, under anaerobic conditions, the "total" heat is equal (or approximately equal) to the "initial" heat, so that in this case the anaerobic delayed heat is practically non existent

Method

The experimental details have been described in the first paper of this series If a muscle, placed on a thermopile, and in good temperature equilibrium with it be stimulated by a succession of shocks, the *total* heat, up to the time when the galvanometer comes completely to rest again is given by the area of the deflection-time curve Part of this total heat is due to "basal" resting processes, in no way connected with the contraction, part to those reactions which lead to the increment in the resting heat rate after stimulation, also not to be debited against the contraction process It is necessary, therefore, to adopt a "base-line" from which to measure the *total heat due to activity* In fig 2 the full line representing galvanometer deflection started from zero in the diagram 6 minutes after 2 minutes' stimulation (65 twitches) it returned not to zero but to 10 mm where it would have remained indefinitely The base line, therefore is drawn horizontally backwards from 8 minutes to 2 minutes at a constant height of 10 mm, and then a sloping line is drawn from 10 mm to the origin The total heat due to activity is measured by the area of the curve above this base line In fig 2 the broken line represents the thermal response of the same muscle to a second 2 minutes' stimulation (63

22.12.27.

Tl/H = 4·72. ΣTl/H = 4·75.

ΣTl/H = 4·51. Tl/H = 4·55.

3.1.28.

Tl/H = 5·37. ΣTl/H = 4·81. Tl/H = 5·27.

7.10.27.

Tl/H = 4·25.

ΣTl/H = 4·42. ΣTl/H = 4·44.

Tl/H = 4·30.

Fig. 1.—Isometric records of twitches in three experiments.

22.12.27.—Test-shocks: Series I: Series II: test-shocks.

3.1.28.—Test-shocks: Series I: test-shocks.

7.10.27.—Test-shocks: Series I: Series II: Series III: test-shocks.

Above or beneath each group or series is printed the corresponding value of the isometric heat-coefficient, Tl/H or ΣTl/H. In the former case H is initial heat; in the latter case H is total heat.

twitches). This started from 10 mm., the level reached at the end of the first series, and returned finally to 20 mm. The base-line, as before, consists of a horizontal part over the later period, and a sloping part over the earlier period. The increment in resting heat-rate is supposed to occur uniformly down the interval of stimulation.

The total heat due to activity can be measured with great accuracy. The initial base-line is steady to 1 mm. or less. The galvanometer deflection is recorded at 6-second intervals, and is of the order of 500 mm. at its maximum: it is impossible to detect the divergence of the observed points from a curve drawn on the scale of fig. 2. The final base-line is steady to 1 mm. The

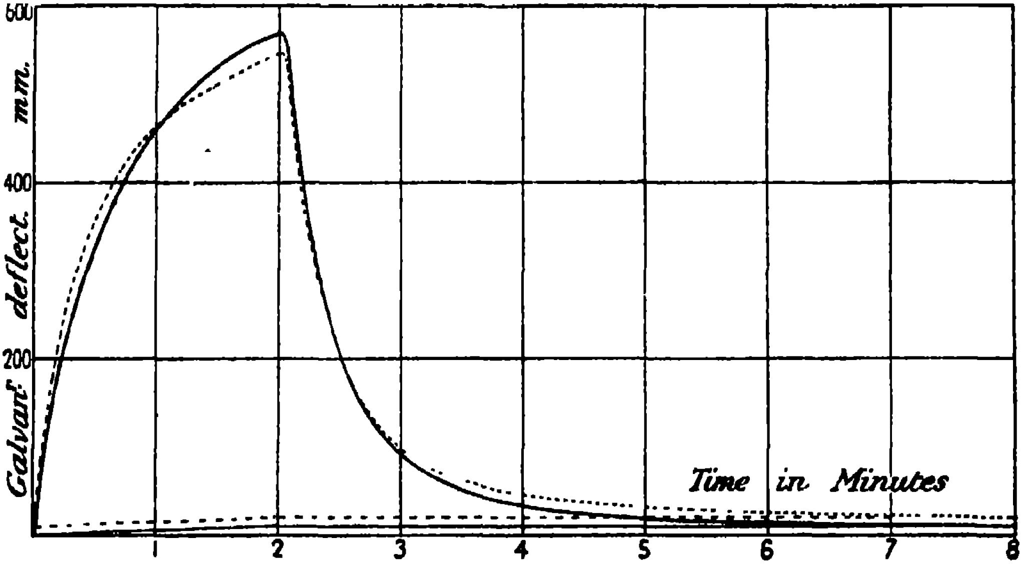

Fig. 2.—Records of galvanometer deflection in Series I and II of experiment of 22.12.27 (see fig. 1). Muscle in pure nitrogen. Two minutes' stimulation. Base-lines drawn beneath. Full line—Series I, 65 twitches. Broken line—Series II, 63 twitches.

calibration required to turn millimetre-minutes into gramme-centimetres is accurate to within 1 or 2 per cent. The total heat-production of muscle can, in fact, be determined in this way with an accuracy unequalled by any other method, and can be compared with the initial heat measured by the deflection in a single twitch. For the comparison it is necessary to calculate the heat per unit of tension developed. For this purpose the isometric response is simultaneously recorded as in fig. 1, the sum of the tensions ΣT developed in a series being determined and multiplied by l, the length of the muscle between the electrodes (the part calibrated). The quotient ΣTl/H represents the total tension-length developed per unit of *total* heat set free.

It is possible, if desired, to analyse the curves of fig. 2 by means of a control curve of deflection obtained by 5 seconds of uniform heating of the dead muscle, and so to exhibit the actual heat-rate throughout the stimulation. Fig. 3 shows such an analysis for a case in which a delayed anaerobic heat-production does seem definitely to have occurred, viz., the experiment of 3.1.28 of fig. 1, and Table I. The twitches are of uniform height, but the rate of heat-production rises somewhat during the period of stimulation, and does not drop immediately to zero when the latter ends. Table I shows a delayed anaerobic heat of 11 per cent.--determined in another way--for this experiment. Several experiments, analysed in this manner, have shown a similar, though smaller, delayed heat. There is no doubt in such cases of its occurrence, but its amount is so small on the average--see Table II--that it may be said practically to be absent. The analysis of the deflection records in this way gives information less reliable, and less easily interpreted, than the method now to be described.

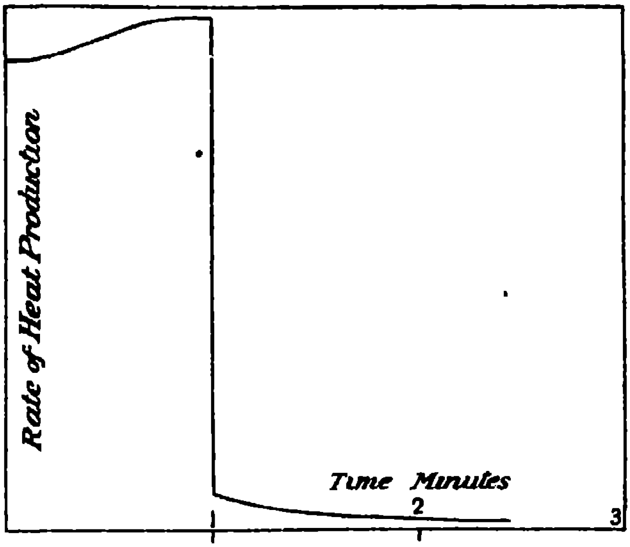

FIG. 3.—Analysis of the rate of heat-production during one-minute anaerobic stimulation with break shocks. For mechanical response, 31 twitches of uniform height, see fig. 1, experiment of 3.1.28.

For the comparison of initial and total heat it is necessary to employ several single twitches before and after the series in which total heat is measured, and to record (*a*) the deflection, giving the initial heat, and (*b*) the tension in the isometric response. The mean value of Tl/H in these " test-shocks " is then calculated, and represents the tension-length developed per unit of *initial* heat set free. The average of Tl/H before and after the series is then compared with ΣTl/H in the series. If they be equal, the initial heat is equal to the total heat; if the former be the greater, the initial heat is less than the total heat, and the difference must be due to a delayed anaerobic heat-production. The value of the initial heat can be determined with considerable exactness in a twitch, the deflection being of the order of 50 or 60 mm. on the scale, readable to ½ mm., and the calibration accurate to within 1 or 2 per cent. Thus the comparison of initial with total heat by this method leaves little margin for error, and allows a definite decision to be reached as to the existence, or otherwise, of delayed anaerobic heat in a series of twitches.

The assumptions made in the comparison are simple and readily admitted.

A.—The maximum deflection of the galvanometer after a single twitch is taken as the initial heat; the deflection occupies only a few seconds, and many analyses of photographic records of the thermal response to a short stimulus have shown that the maximum deflection is little, if at all, affected by delayed heat.

B.—The area of the deflection-time curve has been taken as the total heat; the basis of this assumption has been given in the first paper of this series. The total heat-production associated with activity is certainly complete by the time the galvanometer has become steady again, and the curves have always been followed to complete steadiness.

C.—Each unit in a series of twitches at 2- or 3-second intervals has been taken as comparable with a single isolated twitch. A twitch at 15° to 20° C. occupies only a few tenths of a second, and an interval of 2 or 3 seconds is relatively so long that no appreciable interference would be expected between successive units of the series. A careful examination of fig. 1 shows a close return to the base-line between twitches. Occasionally a very obvious *treppe*, or fatigue, or contracture, sets in during a series: this makes comparison doubtful, and such cases have been discarded. Only those experiments have been taken as significant in which the initial test-shocks, the final test-shocks, and the units of the series were similar. In the three sets of records given in fig. 1 this similarity obviously exists. In such cases it would seem unlikely that the units of the series may not be treated as comparable with the individual twitches before and after.

D.—It is assumed that oxidative recovery has been eliminated. In most cases purified nitrogen (less than 0·02 per cent. O_2) has been employed, sometimes with cyanide vapour added. In three cases cylinder nitrogen (0·7 per cent. O_2) was used, but it happens that in these the total heat came out to be very slightly *less* than the initial heat, an effect which could not possibly be due to oxidation.

Results.

In Table I three experiments are described in detail In fig 1 the mechanical records of these are reproduced, and in fig 2 the thermal response of the galvanometer for the first experiment only In fig 1 the three experiments are given separately below their dates In each the initial test shocks came first then the series (one or more), and then the final test shocks Above or beneath each set is written the value observed of Tl/H, or of $\Sigma Tl/H$, as appropriate In the first experiment (22 12 27) the total heat and the initial heat are, on the average, precisely equal, in the second (3 1 28) the total heat is 11 per cent greater than the initial heat, in the third (7 10 27) it is 5 per cent less In fig 2 the two curves differ slightly in shape, but in a manner corresponding to their mechanical records (fig 1), and their two areas are equal.

Table I

Experiment of 22 12 27—Phosphate Ringer, 20 mgr. per cent. P, *p*H 7 6, for 33 minutes then oxygen Two series, total 124 shocks, in oxygen, with complete recovery Pure nitrogen after $2\frac{1}{4}$ hours in oxygen After 20 to 30 minutes in nitrogen test shocks, fig 1, Tl/H = 4 72 From 47 to 54 minutes galvanometer steady to $\pm \frac{1}{4}$ mm Then Series I in N_2, 65 shocks, mechanical response fig 1, galvanometer record fig 2, full line H = 1135 mm sec = 1120 grm cm ΣT = 1900 grm, l = 2 8 cm, $\Sigma Tl/H$ = 4 75 After Series I the galvanometer remained displaced 10 mm from its previous position, owing to the increment in resting heat-rate Then Series II in N_2, 63 shocks, mechanical response fig 1, galvanometer record fig 2, broken line H = 1120 grm cm, ΣT = 1805 grm, $\Sigma Tl/H$ = 4 51 Further steady displacement f galvanometer of 10 mm Then four test shocks in N_2, Tl/H = 4 55

Summary—Twitches before and after, Tl/H = 4·72 and 4 55, mean *4 63*
Series I and II, $\Sigma Tl/H$ = 4 75 and 4 51, mean *4 63*
Anaerobic delayed heat *0* per cent

Experiment of 3 1 28—Phosphate Ringer, 15 mgr per cent P, *p*H 7 2, pure nitrogen running through for 24 minutes, then Ringer replaced by cyanide nitrogen 6 test shocks, Tl/H = 5 37 Then series of 31 shocks in 1 minute, see fig 1 H 737 grm cm, ΣT = 1304 grm, l = 2 72 cm $\Sigma Tl/H$ — 4 81 Finally, 6 test shocks, Tl/H = 5 27

Summary—Twitches before and after, Tl/H = 5 37 and 5 27, mean *5·32*
Series, $\Sigma Tl/H$ = *4 81*
Anaerobic delayed heat + *11* per cent.

Experiment of 7 10 27—Phosphate Ringer, 15 mgr. per cent. P, *p*H 7 5, for 3 hours Then in oxygen, 41 twitches and complete recovery Then in cylinder nitrogen containing 0 7 per cent oxygen. 6 test shocks, Tl/H = 4 25 then 10 shocks not recorded then Series I, 20 twitches, H = 370 grm cm, ΣTl = 1710 grm cm, $\Sigma Tl/H$ = 4 62 then Series II, 79 twitches, H = 1570 grm cm, ΣTl = 6950 grm cm, $\Sigma Tl/H$ = 4 42 then Series III, 40 twitches, H = 785 grm cm, ΣTl = 3480 grm. cm, $\Sigma Tl/H$ = 4 44 finally, 3 test shocks, Tl/H = 4 30.

Summary.—Twitches before and after, Tl/H = 4 25 and 4 30, mean *4 27½*
Series I to III, $\Sigma Tl/H$ = 4 62, 4 42, 4 44, mean *4 49.*
Anaerobic delayed heat — 5 per cent.

The results of 11 experiments of this type are summarised in Table II. The experiments were all satisfactory from the point of view of *treppe*, contracture and fatigue, and of similarity of initial and final test-shocks to the units of the series. Individual variations are seen to occur, but not in any regular way.

Table II.—Summary of Experiments comparing Initial and Total Heat under Anaerobic Conditions.

The values given are of Tl/H (test-shocks) or ΣTl/H (series).

The figures in brackets refer to the number of twitches in a series or group.

The delayed heat is calculated from the ratio of Tl/H (twitches) to ΣTl/H (shocks), subtracting unity from the result and expressing as a per cent.

Date.	Test-shocks.			Series.				Delayed Heat.
	Before.	After.	Mean.	I.	II.	III.	Mean.	
								Per cent.
4.1.28	6·97 (8)	7·47 (8)	7·22	7·24 (63)	—	—	7·24	0
20.12.27	7·2 (3)	7·4 (4)	7·3	7·3 (62)	—	—	7·3	0
22.12.27	4·72 (5)	4·51 (4)	4·61	4·75 (65)	4·51 (63)	—	4·63	0
12.10 27	3·48 (5)	3·67 (4)	3·57	3·67 (58)	—	—	3·67	− 3
Ditto after recovery	3·33 (3)	3·52 (3)	3·42	3·52 (59)	—	—	3·52	− 3
6.2.28	4·34 (8)	4·49 (3)	4·41	4·60 (63)	—	—	4·60	− 4
7.10 27	4·25 (6)	4·30 (3)	4·27	4·62 (20)	4·42 (79)	4·44 (40)	4·49	− 5
25.1.28	6·25 (9)	6·20 (4)	6·22	5·74 (32)	—	—	5·74	+ 8
21.3.28	7·67 (4)	—	7·67	6·98 (51)	—	—	6·98	+10
14.10.27*	—	—	3·67 (6)	4·05 (79)	4·11 (159)	—	4·08	−10
3.1.28	5·37 (6)	5·27 (3)	5·32	4·81 (31)	—	—	4·81	+11
7.1.28	6·03 (5)	5·80 (6)	5·91	5·25 (31)	—	—	5·25	+13
							Mean .	+ 1·4

* Two series with test-shocks between.

They must presumably be due, partly to experimental error, but mainly to unavoidable irregularities in the behaviour of the muscles. The average result, however, is clear. As the mean of 12 complete sets of observations on 11 different muscles, the total heat is found to be only 1·4 per cent. greater than the initial heat. It may be concluded that *in a series of twitches there is little or no delayed anaerobic heat.*

This result is of importance, partly in connection with the "balance sheet" of the energy changes due to chemical processes involved in activity, partly in reference to recent assertions by Embden and his colleagues as to the place of lactic acid in contraction. The former will be considered in a subsequent paper of this series, in which recent experiments on oxidative recovery after a series of twitches are described. The latter will be dealt with here.

Discussion.

On various occasions during the last few years Embden and his colleagues have attacked what they describe as the "Meyerhof-Hill theory" of muscular contraction. In 1926 Embden, Hirsch-Kauffmann and Deuticke (4) claimed to have proved that a considerable proportion of the lactic acid liberated as the result of a tetanic stimulus is set free some time *after*, and not *during*, activity. Their experiments were repeated by Meyerhof and Lohmann (5), who found that the delayed liberation of acid is due solely to the excessive stimulation, the "electrocution," of the muscle. Tetanised through its nerve, or directly with a reasonable strength of current, a muscle liberates no measurable amount of lactic acid after its contraction is over. Furusawa and Hartree (3) confirmed the existence of this result of excessive stimulation;* employing "an extra strong stimulus of short duration," and allowing for its physical heat-production, they found "a relatively enormous output of delayed heat, the total extra heat produced during a period of two or three minutes being well in excess of the initial heat itself." Clearly there was no real evidence for a delayed lactic acid production normally occurring.

Undismayed by this criticism, the validity of which they appear to admit, Embden, Lehnartz and Hentschel (6) repeated the attack on the "Meyerhof-Hill theory" in another form in a second paper, in which they concluded that a considerable part of the lactic acid set free in a series of individual twitches is liberated in the intervals between them, and not during the contractions themselves. This statement was challenged by Meyerhof and Schulz (7), who showed that the experimental results on which their conclusion was based were vitiated by important technical errors. For these the paper by Meyerhof and Schulz may be consulted.

The logic, moreover, by which Embden and his colleagues proceed would seem to be at fault. It will be shown below that a method such as they adopted could not possibly, under any conditions, throw light on the question of whether the lactic acid is liberated during, or after, the contraction. For that purpose other methods are required, and the experiments described above in which the total heat is shown to be equal to the initial heat, in a muscle without oxygen, would appear to leave no doubt as to the answer. Unless we suppose that lactic acid can be liberated without any corresponding heat-production—an alternative which need not be discussed, since the mere neutralisation of the acid produces heat—we are forced to the conclusion that the "total" lactic acid is equal to the "initial" lactic acid, and that "*delayed*" *lactic acid formation does not occur*, except in a muscle subjected to excessive stimulation.

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* So far as evidence of delayed anaerobic heat can be found in Tables I and II, it is probably to be attributed to the same cause, the excessive stimulation of a small proportion of the muscle-fibres—those in immediate contact with the electrodes where the current lines are most dense.

The procedure adopted by Embden, Lehnartz and Hentschel (6) was as follows: a pair of upper-leg muscles from a frog were given 140 to 270 shocks in 30 minutes, one of the pair (A) being in oxygen, the other (B) in hydrogen. They examined the ratio—

$$\frac{\text{Lactic acid (B)} - \text{Lactic acid (A)}}{\text{Oxygen disappearance (A)}}.$$

If (i) the two series were comparable in respect of their mechanical responses, which presumably they are not owing to greater fatigue in (B), and (ii) the amount of oxygen dissolved in the muscle (A) were negligible, which—as shown by Meyerhof and Schulz—it is not, then the ratio would allow a calculation of the "oxidative quotient," as that term is defined by Meyerhof, viz., the ratio of lactic acid removed to lactic acid (or its equivalent of carbohydrate) oxidised. They found the oxidative quotient, calculated from their experiments, to vary from 1·6 to 11·8, having a mean value of 7 and an average difference from the mean as high as 2·4. It may be remarked that Meyerhof and Schulz (7) in a repetition of these experiments, with proper experimental precautions, found a mean oxidative quotient of 4·7, a value similar to that determined in a variety of other ways. The high values of their quotients Embden and his colleagues explained by the hypothesis that "during activity in hydrogen more lactic acid is formed," and they concluded that "the assumption underlying the Meyerhof-Hill theory—namely, that the total energy set free during contraction can be ascribed to the exothermic transformation of carbohydrate into lactic acid and the neutralisation processes following thereon—can no longer be maintained."

Various experiments—for example, those by Weiszäcker (8) and by Hartree and Hill (9)—which are confirmed by observations, to be recorded in a later paper of this series, have shown that the initial heat (contraction and relaxation) is the same, for a given mechanical response, in the presence and in the absence of oxygen. From this Embden and his colleagues conclude that the lactic acid formation in the initial phase is the same both in oxygen and in hydrogen. Hence, if the muscle in hydrogen liberates more total lactic acid, that substance must be produced after and not during the contraction. In this case a curious paradox arises, which Embden, Lehnartz and Hentschel apparently did not notice. If, in hydrogen, lactic acid is liberated after the contraction is over, and if this delayed lactic acid production is part of a normal process, it must presumably occur also in a muscle hanging in an atmosphere of oxygen, being followed later on in the latter case by the reactions causing an oxidative removal of the acid. There would seem no reason why a process occurring in the one case should not occur also in the other; the conditions in the interior of the muscle are similar in both, since during stimulation in oxygen the interior of the muscle is in a state of considerable oxygen-want, as shown by the fact that lactic acid accumulates in it. If, however, a delayed lactic acid formation occurred in either case, recovery being possible only in one, the *oxidative quotient would remain unchanged*, so that their high value of the oxidative quotient cannot be explained in this way.

It is necessary, if we adopt the explanation of Embden and his colleagues, to assume that there is a delayed lactic acid formation in the interior of a muscle whose *surface* is in contact with hydrogen, and that there is no such delayed lactic acid formation in the interior of a muscle whose *surface* is in contact with oxygen. The difficulty of such an assumption would appear to invalidate their explanation, even if their experimental results were admitted. It is obvious, indeed, that no decision of the question of whether there is, or is not, a delayed lactic acid formation after a contraction can be arrived at by experiments relating to the oxidative quotient, since, on any reasonable hypothesis, a *delayed lactic acid formation would also require oxygen for its reversal, and therefore would cause no change in the oxidative quotient.* It is clear that a decision can be reached only by studying the lactic acid formed during contraction alone, and comparing that with the total lactic acid liberated during contraction and in a subsequent period of suitable duration. Since, however, the lactic acid formed in a single twitch is much too small to be measured by chemical means, the only way of deciding the matter, in the case of a single twitch, is by experiments on the heat production, and the results recorded above appear to show that no lactic acid formation occurs after the processes of contraction and relaxation are complete.

Summary.

1. A method is described by which, in the absence of oxygen, the "initial" heat-production in a muscle twitch can be compared with the "total" heat-production. The two are found to be very nearly equal.

2. Hence, in a muscle twitch, there is little or no delayed anaerobic heat-production.

3. Since lactic acid cannot be formed without heat-production, it follows that there is no delayed lactic acid formation; the lactic acid must appear entirely during contraction and relaxation.

4. The contrary conclusion of Embden, Lehnartz and Hentschel, the experimental basis of whose work has been criticised by Meyerhof and Schulz, is shown to rest on imperfect reasoning; the method they adopted could not lead to a decision of the question.

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The Recovery Heat-Production in Oxygen after a Series of Muscle Twitches.

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Experiments determining the recovery heat-production in oxygen after a short tetanic stimulus have been described on various occasions, *e.g.*, by Hartree and Hill (1) (2) and by Hartree and Liljestrand (3). In employing the results of these experiments to calculate the "oxidative quotient for lactic acid" as defined by Meyerhof (see, for example (4), p. 567), viz.:

$$\frac{\text{(Lactic acid removed in recovery)}}{\text{(Lactic acid — or carbohydrate equivalent — oxidised)}}$$

a complication arises in respect of the anaerobic delayed heat (see 1, 5 and 6).

It has been assumed that in calculating the ratio,

$$\frac{\text{(Heat in oxygen)}}{\text{(Heat in absence of oxygen)}}$$

the denominator should include not only the initial, but the delayed anaerobic heat. The uncertainty as to the nature and origin of the latter has introduced a certain element of doubt into the calculation, and the result of the preceding paper, showing that in a series of separate twitches there is little or no anaerobic delayed heat - and, therefore, no delayed lactic acid production--has made it desirable to determine the recovery heat also for that case

The experiments to be described below have been devoted to this purpose, and have given a mean ratio of oxidative to anaerobic heat of 2·07, which leads to an "oxidative quotient" of 4·81, a value extremely close to the latest determination, 4·7, made by Meyerhof and Schulz by a different method. The methods employed have been, in all respects, the same as those described in previous papers of this series. The heat in a succession of twitches, in oxygen or nitrogen, has been determined from the area of the deflection-time curve of the galvanometer, the isometric response being simultaneously recorded on a drum. The total heat for a given isometric response in oxygen, up to complete recovery, has been compared with the total heat in nitrogen: the ratio of these two heats gives the quantity required

In fig. 1 the lower record is of 51 isometric twitches in 2 minutes 40 seconds in oxygen; the upper record, which is almost precisely similar to it, is of 51 isometric twitches of the same muscle an hour later in pure nitrogen.

51 twitches in nitrogen.
$\Sigma Tl/H_N = 6\cdot 98$.

51 twitches in oxygen.
$\Sigma Tl/H_O = 3\cdot 03$.

FIG. 1.—Two similar series of isometric twitches, in oxygen (lower record) and in nitrogen (upper record). Read from left to right. H_O is the total heat in oxygen (including recovery): H_N is the total heat in nitrogen. For simultaneous heat records, see fig. 2.

In fig. 2, curve (A) represents the galvanometer deflection due to the heat-production in oxygen; curve (B), that caused by the heat-production in nitrogen. Curve (B) reaches a constant level in less than 10 minutes: curve (A) continues to fall to its base-line for 40 minutes. The last part of curve (A) is inset on a different scale, five times the scale of deflection, one-fifth the scale of time; this device makes the fall more obvious, and enables the last part of the area of the curve to be determined with greater accuracy. As pointed out in the second paper of this series, the resting heat-rate of a surviving muscle in oxygen continues slowly to fall for long periods; after 40 minutes the galvanometer deflection corresponding to this heat-rate is 8 mm. less than it was at the beginning.* Consequently the base line is drawn sloping. On the original scale this can barely be detected: in the inset it is 25 times as great, and is obvious. The total heat in oxygen H_O is given by the area of curve (A) above its base-line. In this case $\Sigma Tl/H_O = 3\cdot 03$. The resting heat-rate in nitrogen, on the other hand, receives a permanent increment as the result of stimulation. The deflection is finally 20 mm. more than it was initially. This increment, as described in previous papers, is supposed to occur uniformly during stimulation: consequently the base-line consists of two parts, a sloping one during the period of stimulation, and a horizontal one afterwards. The total heat in nitrogen, H_N, is given by the area of curve (B) above its base-line. In this case, $\Sigma Tl/H_N = 6\cdot98$. The ratio of these

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* In the figure the line of zero deflection is not galvanometer-zero, but is chosen to coincide with the position at the beginning of each experiment.

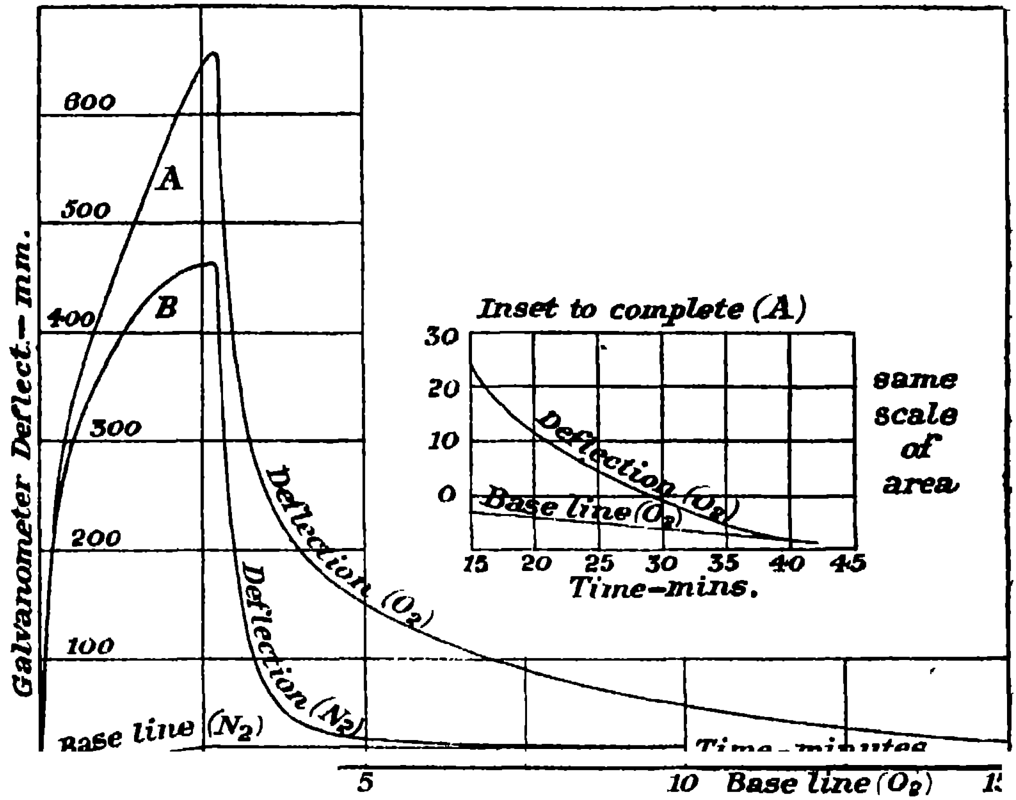

FIG. 2.—Galvanometer records of heat-production in the two series of muscle twitches of fig. 1 (A) in oxygen, (B) in nitrogen, each 51 twitches in 2 mins. 40 secs. The total heat is given by the area of each curve above its appropriate base-line. Inset, the later part of curve (A), reduced in scale five times horizontally, increased five times vertically.

two quantities, viz., $6\cdot98/3\cdot03$, is $2\cdot30$, which represents the "recovery ratio for heat."

It will be seen that curve (A) begins to diverge from curve (B) in 15 or 20 seconds, and goes far above it during the period of stimulation. It is possible by constructing a "control" curve for a uniform heating of 5 seconds' duration to analyse such curves, and fig. 3 represents an analysis (by my colleague, Mr. W. Hartree) for the case of 73 shocks applied to a muscle in 4 minutes in oxygen. The original deflection-time curve is not given. The line of dots (B) reproduces the heights of successive twitches in the series. Curve (A) represents the rate of total heat-production, as yielded by analysis, dropping instantly at the end of stimulation to a level representing recovery-heat only. The amount of sudden drop is a measure of the rate of "initial" heat-production towards the end of the period of stimulation. The level of curve (A) at the beginning, before the recovery process has begun, gives the rate of "initial" heat-production at the beginning. The two points so determined allow us to draw in the broken line (C), giving the rate of "initial" heat-production: (C) runs closely parallel to (B), the mechanical response. The slight drop in (A) at the beginning is fortuitous: the mechanical response showed a similar fall at the beginning, with a rise later on. The recovery process begins to cause a rise in curve (A) in about 25 seconds: it takes this time for the recovery process to work up to an appreciable magnitude.

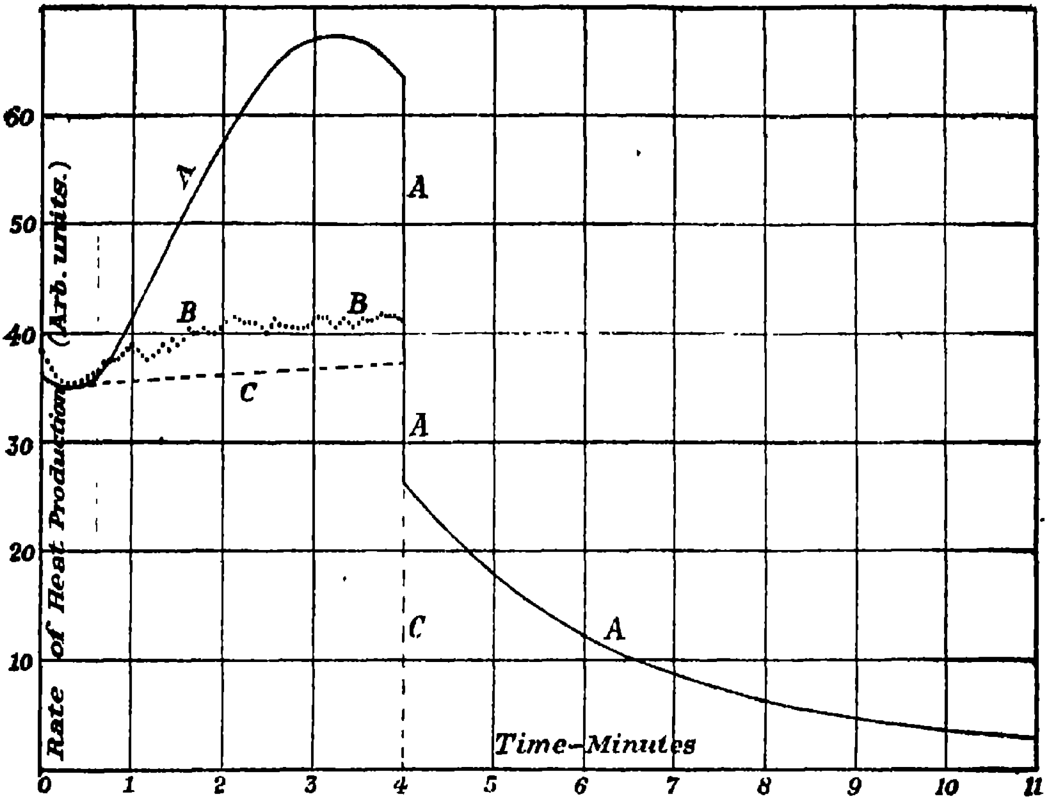

Fig. 3.—Analysis of the rate of heat-production (initial plus recovery) during and after a series of 73 muscle twitches in oxygen. At the end of stimulation the curve drops instantly since the initial heat is no longer being produced. The later stages, to complete recovery, are not shown. Curve (A), rate of total heat-production; curve (C) deduced rate of initial heat-production; excess of (A) over (C), rate of recovery heat-production. Curve (B), height of isometric twitches of series. The existence of a maximum in curve (A) is attributed to the exhaustion of the dissolved oxygen, and an insufficient supply by diffusion.

An interesting feature of the curve is that it reaches a maximum in about 3¼ minutes. At this point, presumably, part of the muscle ceased to be supplied with oxygen, and had exhausted that originally dissolved in it: the increased oxygen-usage of the outer layer prevented sufficient from reaching the interior by diffusion. From that stage onwards the course of the curve is determined largely by the speed of diffusion of oxygen inwards.

Eight experiments have been performed in this way, on muscles in good condition and exhibiting a low resting heat-rate in nitrogen. If the series in oxygen and in nitrogen were not strictly comparable, the experiment was discarded. Results are given in Table I.

Table I.—Determination of the Recovery Ratio for Heat-Production.

The values given are for $\Sigma Tl/H_O$ and $\Sigma Tl/H_N$ as appropriate.

The "ratio" is obtained by dividing the latter by the former. Figures in brackets refer to the number of twitches in the series. The order of the experiment is followed.

Experiment of	2.1.28.	Oxygen, 2·15 (30), 2·15 (29); oxygen-HCN, 5·12 (29) Ratio, *2·38.*
,,	21.3 28.	Oxygen, 3·03 (51); nitrogen, 6·98 (51). Ratio, *2·30.*
,,	12.10.27.	Oxygen, 1·61 (56), 1·69 (56); nitrogen, 3·67 (58), 3·52 (59). Ratio, *2·18.*
,,	19.10.27.	Oxygen, 1·69 (60), 1·78 (80); nitrogen, 3·69 (60). Ratio, *2·13.*
,,	7.10.27.	Oxygen, 2·17 (36); nitrogen, 4·62 (20), 4·42 (70), 4·44 (40); Oxygen, 2·35 (38). Ratio, *1·99.*
,,	20.12.27.	Oxygen, 3·71 (62); nitrogen, 7·30 (62). Ratio, *1·97.*
,,	22.12.27.	Oxygen, 2·44 (62), 2·44 (62); nitrogen, 4·75 (65). Ratio, *1·95.*
,,	22.3.28.	Oxygen, 4·08 (77), nitrogen, 6·72 (78). Ratio, *1·65.*
		Mean value of ratio = *2·07.*

The mean value of the ratio of oxidative to anaerobic heat, for a given mechanical response, is 2·07. The variation is rather large, much too large to be attributed to errors of observation or adjustment. It is probably due to a change in the isometric heat-coefficient Tl/H during the course of an experiment. Such changes do occur, although in a random way, and it is implied in the procedure adopted that Tl/H, where H is the initial heat, is the same in both series. The average divergence from the mean value is ± 0·18, which would give a probable error of the mean of about ± 0·05, about 2½ per cent. The accuracy is sufficient for the purpose.

We may now calculate the "oxidative quotient." Let us assume that the production of 1 gram of lactic acid in the initial phase of contraction is accompanied by the liberation of 385 calories. Then the total heat in contraction and recovery is 385 × 2·07 = 797 calories. The oxidation of 1 gram of dissolved glycogen $(C_6H_{10}O_5)_n$, see Slater (7), yields 3,836 calories. The ratio of these quantities is 4·81, which is the "oxidative quotient." In the latest experiments by Meyerhof and Schulz (4, p. 568), involving measurements of lactic acid formation and oxygen consumption, the mean value is 4·7. No better agreement could be desired. The consistency of such results makes it difficult to imagine that lactic acid will be deposed from its key position in the theory of muscular contraction.

The experiments described represent the most accurate way of approaching the problem. Another way, however, is possible; the anaerobic and the oxidative processes can be completely separated from one another. A muscle is caused to give a series of twitches in nitrogen and its total anaerobic heat-production is measured. Oxygen is then admitted to the thermopile chamber, sufficiently slowly to cause no temperature disturbance, and the recovery process ensues. The experiment is a dramatic one to witness. Within a few

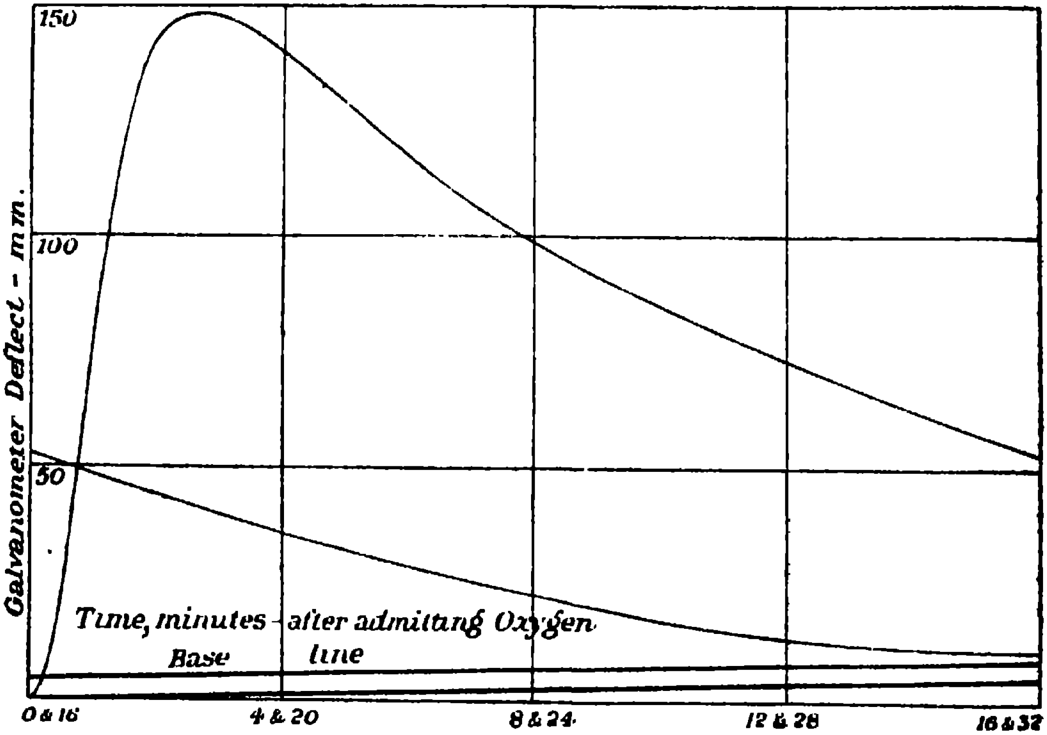

Fig. 4.—Galvanometer deflection recording heat liberated on admitting oxygen to a muscle previously stimulated in nitrogen. The total heat is given by the area of the curve above its base-line. Heat liberated in 76 twitches in nitrogen 11100 gr.-cms. per grm.: heat liberated in recovery 11480 gr.-cms. per grm.

Note.—To save space the curve and its base-line are drawn twice across the same diagram.

seconds of admitting the oxygen the galvanometer spot begins to move, and it remains away from its base-line for a long period. The course of the deflection is shown in a typical experiment in fig. 4, where about 0·27 calorie per gram was liberated in recovery. The curve—to save space—is drawn twice across the same diagram. The base-line, joining the initial to the final level, also appears twice. The total heat liberated in recovery is given by the area of the curve above the base-line. Such a curve could be analysed by means of a control curve made, by artificial heating, to exhibit the rate of heat-production throughout recovery; the result, however, would depend mainly upon the speed of oxygen diffusion into the interior of the muscle, a matter of little interest in the present connection, only the total heat, therefore, has been calculated. The results of three experiments are given in Table II.

Table II.—Heat-Production in Separate Recovery.

The ratio given is of (recovery heat + initial heat) to (initial heat).

Experiment of 20.3.28.	70 twitches in nitrogen.	H = 11100 gr.-cms. per grm.
	Recovery in oxygen.	H = 11480 ,, ,,
		Ratio, *2·03.*
,, 22.3.28.	78 twitches in nitrogen	H = 9040 gr.-cms. per grm.
	Recovery in oxygen.	H = 9500 ,, ,,
		Ratio, *2·05.*
,, 21.3.28.	51 twitches in nitrogen.	H = 6900 gr.-cms. per grm.
	Recovery in oxygen.	H = 11460 ,, ,,
		Ratio, *2·66.*

With such a small number of experiments it would obviously not be just to base any argument on the mean value, which is 2·25. It is clear, however, that this method yields results of the same order of magnitude as the preceding one.*

There is one further point, in connection with the rôle of oxygen in muscular contraction, of which confirmation by improved methods may seem desirable. It was shown by Weizsäcker (8) that the magnitude of the initial heat-production is the same, whether the muscle be in oxygen or in nitrogen. Hartree and Hill (9) proved that the time-relations of the initial heat are unchanged by replacing one gas by the other. During the course of the present experiments a number of observations have been made, in oxygen and nitrogen, on the isometric heat coefficient Tl/H in a muscle twitch, H being the initial heat. The following experiment may be quoted in full :—

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* It has been found that if stimulation be prolonged, recovery is not complete, or is extremely protracted; the recovery mechanism apparently is injured by too much anaerobic activity. This may be related to the degenerative processes induced by anaerobic stimulation (see the second paper of this series).

Experiment of 6.10.27 Six test-shocks in oxygen, Tl/H = *4·82*: 55 shocks in O_2 and recovery: 3 test-shocks in O_2, Tl/H = *4·59*. Six test-shocks in nitrogen, Tl/H = *4·67*: 79 shocks in N_2: 6 test-shocks in N_2, Tl/H = *4·97*. Three test-shocks in oxygen, Tl/H = *5·01*: 30 shocks in O_2: 2 test-shocks in O_2, Tl/H = *4·47*. Eight test-shocks in nitrogen, Tl/H = *4·60*: 60 shocks in N_2: 3 test-shocks in N_2, Tl/H = *4·80*. Thirty shocks in oxygen: 3 test-shocks in O_2, Tl/H = *4·64*. Six test-shocks in nitrogen, Tl/H = *4·65*.

Summary. Mean Tl/H all oxygen observations = 4·71.
Mean Tl/H all nitrogen observations = 4·74

This experiment lasted all day, the muscle maintaining its condition throughout. The response at the end was as great as at the beginning. Fluctuations occur in the coefficient Tl/H, perhaps due to alterations in the alkalinity of the muscle, but on the whole the effect of changing from oxygen to nitrogen is seen to be negligible. The results of four other experiments may be quoted.

Experiment of 7.10.27. Values of Tl/H in O_2, 4·1 and 3·95, mean *4·02*.
Values of Tl/H in N_2, 4·25 and 4·30, mean *4·27*.
" 12.10.27. Tl/H in O_2, 3·24 and 3·28, mean *3·26*.
Tl/H in N_2, 3·48, 3·67, 3·33, 3·52, mean *3·50*.
" 21.3.28. Tl/H in O_2, mean *7·63*.
Tl/H in N_2, mean *7·67*.
" 29.9.27 No calibration. T/H in arbitary units:
In O_2, 4·7, 4·6, 4·7, 4·3, mean *4·6*.
In N_2, 4·5, 5·3, 4·8, 4·9, mean *4·9*.

There is seen to be a tendency for the value of Tl/H in nitrogen to be slightly higher than in oxygen, the heat, per unit of tension developed, to be slightly greater in oxygen, or perhaps the tension per unit of heat to be slightly less. The difference, on the average, is only about 4 per cent., and is probably of no significance. The muscle kept in oxygen, with its recovery process complete and all lactic acid removed, tends to be slightly too alkaline to give its greatest value of Tl/H, while in nitrogen the optimum pH is more nearly approached and the coefficient rises a little. It is clear that, apart from a possible small effect of this kind, the isometric heat coefficient is unaffected by a change from oxygen to nitrogen, so that *the initial process is entirely non-oxidative in nature.*

Summary.

1. Experiments are described in which the total heat set free as the result of a series of muscle twitches in oxygen is compared with the total heat from a similar series in nitrogen. The ratio (heat in oxygen): (heat in nitrogen), has a mean value of 2·07.

2. Since, in the case of a series of twitches, there is little or no delayed anaerobic heat, this ratio can be used directly to calculate the "oxidative quotient for lactic acid" (Meyerhof). The value so calculated is 4·81. This agrees well with the mean value, 4·7, of Meyerhof and Schulz, deduced from experiments involving measurements of lactic acid formation and oxygen consumption.

3. By stimulating a muscle in nitrogen and measuring the heat produced, then admitting oxygen and measuring the recovery heat, it is possible completely to separate the heat-production of the two phases. Such experiments lead to results which are consistent with those described above.

4. The "isometric heat coefficient" of a muscle twitch, Tl/H, where H is the initial heat, is shown to be the same in oxygen and in nitrogen. The initial heat, therefore, is entirely non-oxidative in nature, agreeing with the conclusions of Weizsäcker and others.

The expenses of this research have been borne by a grant from the Foulerton Fund of the Royal Society.

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REPORT

OF THE

GLASS WORKERS' CATARACT COMMITTEE.

The selection by a Departmental Committee of the Home Office in 1906 of diseases which should be added in the Third Schedule of the Workmen's Compensation Bill, 1906, brought into prominence the occurrence of "a form of cataract, commencing in the posterior cortical region of the lens, almost peculiar to persons exposed to the glare and heat" from the furnaces used in glass blowing. The details of this disease at that time available were well summarised in a Report to the Home Office by Dr. T. Morison Legge, H.M. Inspector of Factories (1907).

The Secretary of State wrote to the President of the Royal Society (Lord Rayleigh), asking "whether elucidation of the physical and physiological problems involved could properly be made the subject of an enquiry by a Committee of the Royal Society" (April 7, 1908). The Council of the Royal Society thereupon appointed a Committee "to enquire into and report on the physical and physiological problems involved in the disease known as Glass Workers' Cataract" (June 18, 1908). The Committee consisted of Sir W. Abney, Sir Clifford Allbutt, Dr. H. K. Anderson, Prof. J. Rose Bradford, Dr. G. J. Burch, Sir W. Crookes, Messrs. Marcus Gunn, E. Nettleship, J. H. Parsons, and Dr. A. D. Waller.

At the first meeting held on July 3, 1908, Sir William Abney was elected chairman. A Sub-Committee consisting of Dr. Anderson, Dr. Burch, Mr. Gunn, and Mr. Parsons was appointed to visit some of the principal glass factories; and a Sub-Committee consisting of Dr. Anderson, Dr. Burch, Sir William Crookes, Mr. Parsons and Dr. Waller was appointed to carry out experimental investigations into the problems submitted to the Committee.

In August and September, 1908, the Visiting Sub-Committee inspected glass works at Sunderland, Southwick, Gateshead, and St. Helens, and cases of cataract in glass workers were examined. The thanks of the Committee were subsequently conveyed to the owners of the works, and to Dr. William Robinson and Dr. H. C. Dixon for collecting cases.

The clinical investigation confirmed the existence in bottle makers of a characteristic form of cataract, showing a dense well-defined disc of opacity in the centre of the posterior cortex, not infrequently surrounded by slightly irregular hazy opacities.

The typical condition is common in the makers of heavy glass bottles (beer bottles, etc.), and probably also in the makers of plate glass, though the Sub-Committee had no opportunity of proving this point. It is uncommon in the makers of pressed glass ware, and seems to be quite absent among the makers of flint glass bottles. It is to be noted that the conditions are quite different in the tank furnaces used in the manufacture of beer bottles from the conditions in the pot furnaces used for flint glass bottles and pressed glass. The temperature in the former greatly exceeds that in the latter.

At a later period (1910) Mr. Parsons, during a visit to the United States, took the opportunity of visiting the Salem Glass Works in New Jersey. He reported as follows :—

" The main feature in which these works, which are devoted to the manufacture of bottles, differs from the English bottle works inspected by the Sub-Committee is in the use of machines for blowing and moulding the bottles. By each of these machines, 300 dozen large or 500 dozen small bottles are made in 8 hours. The gathering of the metal is not automatic, but is performed by ' gatherers ' who work in half-hour shifts and are much exposed to the glare of the furnace. The bottles are finished by a ' finisher,' who uses an oil furnace in which he softens the mouths of the bottles and moulds them, but does not apply a ring of fresh ' metal ' as in England.

" The temperature of the tank furnaces is recorded by a Brown electric pyrometer, and a temperature of 2300° F. is aimed at. In one furnace the pyrometer registered 2250° F. at the time of my inspection. I was informed that probably the pot furnaces were nearly as hot as the tank."

He was able to examine ophthalmoscopically the eyes of nine men, two of whom showed slight cataractous changes, but the appearances were not typical of Glass Workers' Cataract. Since this form of cataract is commonest in " finishers," who are the most skilled men and have worked longest at the trade, it is probable that the introduction of blowing bottles by machinery has eliminated much of the danger. It may be stated that in recent years machine-blowing has largely replaced blowing by mouth in England.

The Sub-Committee considered that if the cause of the disease is connected with exposure to light and heat, it might be expected to occur in ironworkers A letter enquiring into this matter was sent to 73 British ophthalmologists and 55 American ophthalmogists, but no evidence of the incidence of this form of cataract in iron workers could be obtained. It was only long afterwards (1921) that indubitable evidence of its occurrence was obtained (*vide infra*).

The Sub-Committee recommended:—

(*a*) Detailed investigation of the physical conditions; and

(*b*) Investigation of the transmission of radiant energy through the media of the eye.

Physical Conditions.

The physical conditions were investigated by Sir William Crookes, and are contained in the following report (April, 1909):—

Accompanied by Mr. Gardiner, my assistant, I visited the Glass Bottle Works of Messrs. Nuttall & Co., St. Helens, and carried on some experiments there, on March 28 and 29, 1909. The object of the visit was to take photographs of the spectrum of the radiation emitted by the molten glass, and thereby gain information as to the rays likely to injure the eyes of the workmen most frequently exposed to the radiations.

When we were there light green bottle glass was being made. The mixture is composed of silica, sodium sulphate, and calcium carbonate or sulphate. The materials are melted in a large fire-brick tank, heated by a flaming mixture of gas and air playing on the surface. The gas is made some distance from the furnace in a "producer," and gas and air are conducted by separate channels to the upper part of the tank, where they mix and burn like a gigantic Bunsen burner, reverberating from the arched roof and heating the glass mixture to the highest degree.

The area of the tank of molten glass is about 82 square yards, and it contains from 300 to 350 tons of the mixture, technically called "metal." There are several such tanks in the works. The tank is divided into two unequal parts by a fire-clay partition, having at the lower part an opening through which the melted glass can flow. The larger portion of the tank has a surface of about 63 square yards. Here the materials are melted together at a high heat. This is called the "melting end," and when the mixture is well fused and homogeneous the molten glass flows through the opening into the "working end" of about 19 square yards, where the heat is less and the glass is in a viscous state. Fire-clay rings of about 18 inches internal diameter and a foot deep float on the surface of the viscid glass, and any scum which is on the surface of the tank is thereby kept from contaminating the surface of the glass inside the ring. One ring floats opposite each working opening, and the workmen withdraw the requisite quantity of glass for each operation from the inner surface of the ring.

The light from the melting end of the tank, viewed through a working opening, was a very brilliant white with a tinge of orange, and it was only with difficulty that the unprotected eye could make out any details. Viewed through dark blue glasses the surface of the metal in the tank appeared a boiling seething mass in constant commotion. The surface in the working end was more easy to see. It was of a bright yellow incandescence, and was comparatively quiet. It is not at all certain what the temperatures are at each end of the tank. Mr. Nuttall told me that approximate measurements taken many years ago with an old form of resistance pyrometer gave the temperature at the melting end at 2200° C., and that at the working end at 670° C. I should not estimate the difference between the two ends to be so great, and I think 2200° C. is too high. It would be of interest for this enquiry to ascertain the actual amount of radiant heat to which the workmen's eyes are exposed, and I regret I had not with me a Fery resistance pyrometer, with which accurate measurements of the temperatures could have been taken, with an error of less than 10° C.

About each opening, especially at the melting end, thin white vapours were noticed, rising and settling on the surrounding cooler parts. A piece of paper held in this vapour instantly ignited. Examined with a hand spectroscope the yellow line of sodium was seen to be brilliant in this vapour, but the light from the molten glass showed a continuous spectrum in which the sodium line was seen only occasionally. On one or two occasions a black line was seen in place of the yellow sodium line, showing a reversal. (This reversal is seen on photographs Nos. 1 and 2.) Some of the condensed vapour was collected from the cool sides of the working opening and examined chemically. It was found to consist principally of sodium and calcium sulphates, with a little sodium chloride.

The spectrograph used for taking photographs of the radiation from the molten glass is the one I described in 'Roy. Soc. Proc.,' vol. 65, p. 237, May, 1899. It has two quartz prisms, each made up of two halves, one half being right- and the other half left-handed, according to Cornu's plan for neutralising the effect of double refraction. The collimating and camera lenses and the double condensers are also of quartz cut in the same fasion. The slit jaws are made of two acute-angled quartz wedges, edge to edge. The refracting prisms are of 60° angle, and each face is 35 mm. by 42 mm. The lenses are 52 mm. diameter and 350 mm. focus. The condensers are plano-cylindrical, one being double the focus of the other. In order to ascertain the exact position of any part of the spectrum I might obtain from the radiation from the molten glass, I took photographs on each plate of an alloy of equal molecular weights of zinc, cadmium, tin and mercury. This alloy gives, throughout the photographic region, lines, the wave-lengths of which are well known.

The instrument was pointed downwards, so as to allow the radiation from the surface of the melted glass to enter the condensers, prisms, and slit along the axis; and to prevent the great heat injuring the spectrograph Mr. Nuttall allowed the opening to be bricked up, leaving a hole a few inches square in the middle. This was covered with an iron plate with a 2-inch hole in it, and over this a quartz plate was fixed.

Wratten and Wainwright's panchromatic films were used. These are sensitive beyond λ 7800 in the ultra-red, and to the highest ultra-violet rays which will pass through quartz. Flexible films had to be used in preference to glass, as they had to follow the curvature of the focal plane. Many preliminary experiments were made to ascertain the extent of spectrum to be recorded, its best position on the films, and the exposures needed. The slit of the instrument was generally placed about 4 feet from the molten surface, and it was found that from 10 to 15 minutes were required to produce a faint image on development.

No. 1 photograph* (see Crookes, 'Phil. Trans. Roy. Soc.,' A, vol. 214, p. 1 (1914)) was taken at the working end of the tank, where the temperature was lower than at the other end. An exposure of 20 minutes was given, the width of the slit being 0·025 mm.

No. 2 photograph was taken in the same conditions as No. 1, but with an exposure of 45 minutes. On each film immediately before the radiation picture was taken, a photograph of the spark spectrum of the quadruple alloy was impressed on the film, in such a position that the two spectra would overlap to a very slight degree. Each photograph is mounted with a coloured drawing of the visible spectrum of daylight below it, so that the rays which enter the eye as light are seen as impressed in the photograph.

No. 3 photograph was taken at the melting end where the heat was fiercest. The width of the slit was reduced to 0·01 mm., and an exposure of half an hour was given.

No. 4 photograph, at the melting end, was exposed for 1 hour.

No. 5 photograph, at the melting end, was exposed for 2 hours.

No. 6 photograph, also at the melting end, was exposed for 3 hours.

It was not found practicable to give longer exposures than this.

While these experiments were going on, experiments at another opening at the hottest end were tried to see if X-rays were to be detected. Sensitive films were wrapped in black paper and then in lead foil in which designs had been cut. These were exposed for varying lengths of time to the radiation from the molten glass as near at it was safe to put them, bearing in mind that the heat might affect the films. On development, no image of any of the stencil designs could be detected on any of the films.

A careful examination of the six photographs shows a general progressive character, the extent of spectrum photographed extending each way as the length of exposure increases. It will be noticed that there is a part of the spectrum round about wave-length 5080 giving a very faint impression. This is most noticeable in No. 2 photograph. This I attribute not to any lack of radiation in that part of the spectrum, but to a deficient sensitiveness in the photographic films used.

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* [These photographs, and the diagram mentioned on the next page, were apparently submitted to the Committee at one of its early meetings. They were not reproduced in the 'Phil. Trans.' paper and do not appear to be now in existence.]

The extent of spectrum embraced in the photographs is shown conveniently in the following tabular form :—

No. 1 photograph, exposed 20 minutes, includes wave-lengths 4520 to 6900.
No. 2 photograph, exposed 45 minutes, includes wave-lengths 4320 to 7200.
No. 3 photograph, exposed 30 minutes, includes wave-lengths 3790 to 7500.
No. 4 photograph, exposed 60 minutes, includes wave-lengths 3640 to 7600.
No. 5 photograph, exposed 120 minutes, includes wave-lengths 3595 to 7700.
No. 6 photograph, exposed 180 minutes, includes wave-lengths 3345 to 7800.

In connection with the above table the following scale for identifying colours with wave-lengths will be useful :—

Wave-lengths 7230 and below = Infra-red.
From 7230 to 6470 = Red.
„ 6470 „ 5850 = Orange.
„ 5850 „ 5750 = Yellow.
„ 5750 „ 4920 = Green.
„ 4920 „ 4550 = Blue.
„ 4550 „ 4240 = Indigo.
„ 4240 „ 3970 = Violet.
„ 3970 and above = Ultra-violet.

Neither of the above tables makes any pretence to scientific accuracy, but they are convenient approximations.

Taking the ordinary limits of visibility to extend from wave-lengths 3970 to 7230, it is seen that with an exposure of 3 hours to the highest heats the strength of impression does not extend much into the ultra-violet, the region of greatest wealth in ultra-violet rays being practically blank. The heat rays at the red end are, however, very strong, and if it is proved that injury to the workmen's eyes is caused by exposure to the radiation from the molten glass, it is, in my opinion, the heat rays, rather than the ultra-violet rays, that are to blame.

I have measured the opacity of the photographic image of spectra Nos. 2 and 6 in many places along the line by means of Mr. Chapman Jones' " Opacity Meter." The results, in the form of curves, are shown on the accompanying diagram.

There is a particular tint of pale green glass used in the Palm House at Kew which has been found to be opaque to the extreme and ultra-red rays (the " scorching " rays) while it scarcely at all interferes with the rays of light. Spectacles or screens made of this glass might be of use in glass works if the workmen would use them.

WILLIAM CROOKES.

April 10, 1909.

Experiments on the transmission of radiant energy by the media of the eye fell into two parts, that of the visible and ultra-violet portion of the spectrum and that of the infra-red.

Luminous and Ultra-Violet Radiation.

Preliminary experiments by Messrs. J. H. Parsons and E. E. Henderson (1909) on the absorption spectra of the cornea, lens, and vitreous of the rabbit with arc light and quartz train showed very considerable absorption of ultra-violet radiation and no selective absorption of luminous radiation. Prolonged exposure of the eyes of albino rabbits to light from "uviol" mercury vapour lamps produced much conjunctivitis ("photophthalmia"). There were marked changes in the epithelium lining of the anterior capsule of the lens. The changes were almost limited to the pupillary area, where the nuclei of the cells were swollen and showed chromatolysis. At the periphery of the pupillary area was a zone in which the cells were smaller and crowded together, the nuclei being more deeply stained. Some nuclear figures indicating mitosis were seen in this area, but these were few in number. No definite opacity of the lens was produced, the radiation from these lamps being relatively feeble. Similar changes were obtained in frog's eyes. One eye of a rabbit was exposed to the naked arc light at a distance of 6 inches for 15 minutes on three successive days. The aqueous of this eye caused slight hæmolysis of guinea-pigs' red corpuscles, the control, non-exposed eye giving no such result.

More exhaustive experiments of a similar nature were carried out by Mr. E. K. Martin ('Roy. Soc. Proc.,' B, vol. 85, p. 319 (1912)). He confirmed the absence of selective absorption of any luminous radiation. He found that the cornea (rabbit) cuts off all rays beyond 295 μμ; the lens cuts off all rays beyond 350 μμ. Radiation between 300 μμ and 400 μμ is therefore transmitted by the cornea and absorbed by the lens, and is therefore capable of inducing changes in that structure. The vitreous shows a band of absorption between 250 μμ and 280 μμ, with a maximum at 270 μμ and ill-defined margins. With the stronger radiation derived from the Kromayer mercury vapour lamp Martin found that corneal opacities were rapidly developed. If these were considerable there were no changes in the lens; but if less intense, changes similar to those observed by Parsons and Henderson occurred in the capsular epithelium. Experiments on the aqueous of rabbits immunised to cat's red corpuscles confirmed Parsons' and Henderson's results, but only if the exposure of the eye to the Kromayer lamp exceeded 1 hour; the unexposed control eye gave a uniformly negative result. These experiments tend to confirm the suggestion of Parsons that the deleterious effect upon the lens may not be due to direct action of the radiation upon the lens but by action upon the ciliary body, setting up a mild iridocyclitis which results in malnutrition of the lens.

Investigation of the transmission of infra-red radiation by the eye was carried out on behalf of the Committee by Messrs. H. Hartridge and A. V. Hill ('Roy. Soc. Proc.,' B, vol. 89, p. 58 (1915)). They proved that heat radiation from 1100 $\mu\mu$ to 700 $\mu\mu$ passes into the eye almost unchecked and a great deal of it reaches the retina. The iris of the ox totally obstructs heat radiation of every wave-length. It therefore absorbs the same percentage radiation as that which reaches the anterior surface of the lens, *i.e.*, roughly 57 per cent. of the heat radiation between 1300 $\mu\mu$ and the visible spectrum. The lens, on the other hand, absorbs of the radiation allowed to reach it through the pupil only about 12 per cent. The authors say that "although an actual coagulation of the lens proteins brought about in the course of time by this small amount of heat radiation is not impossible when the conclusions of Chick and (C. J.) Martin with regard to the physical chemistry of coagulation are considered, yet we think it more likely that the change is due to some interference with the nutrition of the lens caused in some way by the enormous heat-absorbing power of the iris affecting the secretion of the aqueous humour by the ciliary body, as Parsons suggests."

It has since been shown by Vogt and others that short exposure to intense ultra-violet or infra-red radiation can produce opacities in the lens. This is probably a direct coagulation effect, and it is unlikely that the very slow development of cataract in glass workers is due to this process.

Protective Glasses.

The Committee early attacked the problem of protecting the eyes from excessive radiation of all kinds by means of protective glasses or screens. Some preliminary experiments were carried out by Dr. G. J. Burch, as recorded in the following report (April, 1909).—

The object of this research was to find suitable materials for spectacles and for fixed screens to be used in glass works.

With the assistance of my demonstrator, Mr. T. G. Malpas, I have examined—

37 samples of coloured glass.
7 ,, glass coated with a reflecting film.
3 ,, ,, ,, aluminium by rubbing.
2 ,, ,, ,, magnalium ,,
4 ,, wire gauze.
13 combinations of two or more samples.

Inasmuch as it is easy to cut down the light to any required extent in any part of the spectrum, it is obvious that the most suitable substances are those which combine low diathermancy with high transparency.

In the heat-transmission experiments, the radiation from a naked Nernst filament was received on two thermometers A and B, both with blackened bulbs, at such distances from the lamp as to be brought by it to as nearly as possible the same temperature.

A table having been made of simultaneous readings at different temperatures (given by slow small changes of current), the sample to be tested was interposed between the filament and thermometer B and the temperature finally reached by the latter noted. The temperature which would have been reached by B in the absence of the sample was inferred by observing the temperature of A and consulting the table of simultaneous readings, the room temperature being taken.

From these data the heat-transmission coefficient was calculated.

Each result is the mean of several observations extending over an hour or more, and the experiments in all the obviously important cases were repeated by both of us independently.

The Nernst filament was found to be the only available naked source of sufficiently high temperature and sufficient steadiness. Trouble was at first experienced owing to irregular draughts, but this was obviated by enclosing the table in a threefold screen of black gauze, supported by upright posts 18 inches apart fixed between the layers so as to preserve a clear space of at least an inch—2 or 3 inches would be better—between them. This arrangement is curiously efficient. A candle standing on the table could scarcely be made to flicker by blowing at it from outside the gauze, and the two thermometers kept remarkably well together, rising and falling simultaneously with the slight variations of the current. Perfect ventilation and complete absence of draught was thus ensured and the temperature of the enclosure was only about 0·1° C. above that of the room.

N.B.—The sheets of gauze were fastened together underneath the table so that draughts were prevented from blowing between them.

Results.

A pair of neutral-tint spectacles used by one of the workmen at the Ayres Quay Glass Works, Sunderland, was first tested. They transmitted 58 per cent. heat and about 9 per cent. light. This was taken as a basis in determining how far the light could be cut down in practice, for it must be remembered that the men have not only to take glass from the furnace but to manipulate it away from the furnace, where the light is much less strong.

From the Table of Results (p. 201) it appears that cobalt blue is even less efficient in stopping heat than red glass, though the sample tested was slightly thicker than the red.

Table.

Specimen.	Heat transmitted.	Light transmitted.	Thickness.
	Per cent.	Per cent.	cms.
Window glass—Cobalt	66·0	7·5	0·238
,, ,, Green	50·0	7·8	0·214
,, ,, Amber	58·7	58·6	0·298
,, ,, Red	65·5	8·6	0·164
Ordinary flatted plate	74·0	88·3	0·205
Plate glass, colourless	73·5	—	0·682
Spectacle glass supplied by Messrs. Botley & Lewis—			
Cobalt 1	82·4	77·3	0·153
,, 3	79·7	49·0	0·145
,, 3+	76·0	41·6	0·172
,, 4	72·0	32·5	0·138
,, 5	76·0	13·0	0·193
,, 6	72·5	8·5	0·175
Emerald green—2 (iii)	68·7	42·7	0·147
,, ,, 3 (iii)	50·5	18·6	0·192
,, ,, 4 (iii)	62·3	9·1	0·207
London smoke—5 (i) (warm tint)	68·6	15·6	0·194
,, ,, 5 (ii) (greenish tint)	65·0	11·8	0·192
Neutral tint—5* (blue tint)	71·0	14·3	0·256
London smoke—1 (i) (warm tint)	83·7	68·6	0·121
,, ,, 2 (i) (warm tint)	92·7	51·0	0·118
Dr. L. Johnson's spectrum blue	53·2	9·2	0·215
Fienzal's amber	73·5	59·1	0·211
Furnace glass	73·5	2·8	0·209
Bottle green—I (v)	83·1	65·0	0·232
,, ,, II (v)	86·8	60·8	0·262
,, ,, III (v)	85·8	46·3	0·225
,, ,, IV (v)	71·4	39·6	0·244
Supplied by Messrs. James Powell & Sons—			
Blue	43·5	31·0	0·298
Green I	38·0	17·0	0·483
Gilded glass	21·5	0·9	0·371
Green II	46·7	14·0	0·427
Spectacle glass supplied by Mr. J. Pillischer—			
Bottle green	80·2	27·0	0·213
Blue	68·2	9·6	0·203
Amber	74·2	47·3	0·132
Yellow	82·3	75·6	0·150
Pink	79·3	78·8	0·162
Metallic films on glass—			
Galena—1	91·7	60·7	—
,, 2	87·1	43·2	—
Silver—1	86·5	43·8	—
,, 2	47·6	15·5	—
Magnalium	48·7	27·7	—
Aluminium—1	51·8	34·8	—
,, 2	49·4	31·0	—
Aluminium on green glass	37·8	3·4	—
Wire gauze—Iron	68·5	53·0	—
,, Brass	62·0	40·0	—
,, Thickened electrolytically	47·0	19·5	—

Full data relating to combinations of screens are given in the paper.

N.B.—The numbers attached to some of the specimens are not to be regarded as indicating the depth of colour except roughly. In several cases the tints are entirely different.

A piece of amber glass was remarkable in transmitting the same percentage of light as of heat, viz., 58 per cent. Some ordinary green glass transmitted 50 per cent. heat and 7·8 per cent. light. A sample of Dr. Lindsay Johnson's spectrum blue glass transmitted 53 per cent. heat, but only 9·3 per cent. light. A sample of lead glass of a peculiarly clear blue, sent me by Messrs. James Powell & Sons, transmitted 43·5 per cent. heat and 31 per cent. light. A green by the same makers gave 38 per cent. heat and 17 per cent. light. Being lead glass these were much more opaque to X-rays, and the green more opaque also to violet and ultra-violet rays than the other samples.

Metallic Films.

The effect of a metallic film in arresting the passage of heat, utilised with such good effect in the Dewar vacuum flask, suggested the possibility that a film thin enough to be transparent might serve to reflect away a sufficient percentage of the heat, and perhaps without becoming hot.

Dr. Emerson Reynolds kindly lent me two very fine specimens of semi-transparent Galena films on glass. In both the coating was very brilliant and extremely regular. By comparing their heat transmitting power with that of a piece of plate glass of the same thickness, it appeared that the film itself in one case stopped 8·3 per cent. of the heat, the light transmitted being 60·7 per cent. The other film, which was denser, stopped 12·9 per cent. heat, the light transmitted being 43·15 per cent. From some trials made in my laboratory it appeared that the percentage of light transmitted diminished very rapidly with increasing thickness.

We also experimented with silver films. Some half-silvered pieces of clear glass gave results as follows:—No. 1. Heat transmitted 86·5 per cent., light 43·8 per cent. No. 2. Heat 47·6 per cent., light 15·5 per cent.

It would thus appear that the thin film of silver has at first little or no effect in stopping the longer heat rays, and that when thick enough to do so it stops more light than can be spared. Moreover, these thin films seemed unable to stand such hard usage as they would get in practice.

Three pieces of glass were rubbed over with aluminium so as to form on them a fairly even network of close but irregular semi-transparent metallic lines. The effect was remarkable. In round numbers the coated glass transmits only half the heat and one-third of the light transmitted by the same piece before it was coated.

A piece of green glass, which transmitted 53·6 per cent. heat and 7·9 per cent. light, after being rubbed over with aluminium, transmitted 37·8 per cent. heat and 3·4 per cent. light. This, however, is too little, and in this case more light was stopped in relation to heat than when clear glass was used.

Magnalium adheres to glass in the same manner. A piece of clear glass coated with it transmitted 48·7 per cent. heat and 27·7 per cent. light.

Unfortunately, although these coatings will stand hard usage, they interfere somewhat with the definition of objects seen through them.

Wire Gauze.

The ratio of heat transmission to light transmission being very high even in these cases, I determined to try positive screening by wire gauze.

A piece of iron wire gauze transmitted 53 per cent. light and 68·5 per cent. heat. A piece of brass wire gauze transmitted 40 per cent. light and 62 per cent. heat.

The gauze did not seem greatly to matter, the ratio of wire diameter to size of mesh being much the same in all. So we thickened a piece by depositing copper on it electrolytically. It now transmitted 19·5 per cent. light and 47 per cent. heat. It will be observed that in all cases the proportion of heat stopped is much less than that of light. This is no doubt due to secondary radiation; but it is a condition which obtains in practice.

Combinations of Screens.

I then tried the effect of combining various screens, with the following results :—

Powell's blue plus the thickened gauze, close together—
Heat transmitted, 28 per cent. Light, 6·75 per cent.
It made no difference whether the glass or the gauze was next the thermometer.

The same 1 inch apart—
Heat transmitted, 22 per cent. Light, 6·75 per cent.

Powell's blue plus Powell's green, 1 inch apart—
Heat transmitted, 30·2 per cent. Light, 7·3 per cent.

Three pieces of clear glass, 1 inch apart—
Heat transmitted, 52 per cent. Light, 70 per cent.
The same close together—Heat transmitted, 63 per cent.

Three pieces of amber glass, 1 inch apart—
Heat transmitted, 33 per cent. Light, 13·2 per cent.
The same close together—Heat transmitted, 45 per cent.

Three pieces of green glass, 1 inch apart—
Heat transmitted, 36 per cent.
The same close together—Heat transmitted, 50 per cent.

Powell's blue plus two pieces of bottle-green glass, spaced 1 inch apart—
Heat transmitted, 34·23 per cent.

This combination was tried because the Bunsen flame is scarcely visible through it owing to the character of the absorption.

General Conclusions.

If the men can use it, thickened gauze plus Powell's blue glass would be the best combination at present found. But only the men themselves can decide whether the definition is good enough through the gauze, or if it gets too hot so near the face. The same remark applies to the aluminium coatings.

If a fixed screen is practicable, it should consist of several sheets with air spaces between. Probably gauze next the furnace would prevent glass from cracking.

Nearly all the measurements quoted in this paper are due to my demonstrator, Mr. T. G. Malpas, who also made the calculations. I have verified his results by independent observations in the important cases. The light transmission was measured with the flicker photometer, and as the personal element might in dealing with different colours affect the result, I have been careful to compare my readings with his. I find that with low intensities there is a marked difference, but that it disappears if a reasonably strong light is used, and this has accordingly been done in the observations recorded.

I have to thank Dr. Emerson Reynolds for placing at my disposal the galena films above referred to, and for instructions concerning the method of preparing them. And I have also to thank Messrs. James Powell & Sons, both for information concerning the composition of specimens in my possession, and for the samples sent me as most likely in the light of our preliminary experiments to serve the purpose in view.

G. J. Burch.

April, 1909.

Sir William Crookes examined the transmission of radiation by samples of glass—chiefly green glasses—used at various times at the Royal Botanic Gardens, Kew. He then embarked upon the much more exhaustive research of making synthetic glasses by the addition of various metallic oxides to a clear and colourless flux and examining the transmission of radiation through them (see his 'Phil Trans.' paper before quoted). Over 300 tinted glasses were prepared. Of these the composition of the most important, and their transmission of infra-red, luminous, and ultra-violet rays are recorded in his paper. The glass which most effectually obstructs ultra-violet light has since been put upon the market as "Crookes' glass," and has been widely used in spectacles for protection of the eyes, especially in sunny places and at high altitudes. The attempt to introduce protective glasses among glass workers failed, chiefly owing to the innate conservatism of the British workman.

The infra-red and ultra-violet transmissions of several of Crookes' glasses and of other glasses, such as chlorophyll, Fienzal, Euphos, etc., were also determined and published by Hartridge and Hill ('Roy. Soc. Proc.,' B, vol. 89, p. 72 (1915)).

Iron Workers' Cataract.

The work of the Committee was entirely in abeyance during the period of the war, and it was not until 1921 that it could be resumed. Early in that year information was obtained from Messrs. B. Cridland of Wolverhampton, and J. J. Healy of Llanelly, that a form of cataract similar to that found in glass workers occurred in iron puddlers and tin-plate millmen. Cases of the same kind were also reported by Mr. St. Clair Roberts of Worcester in chain makers.

On February 11, 1921, Mr. Parsons and Dr. Morison Legge examined cases of Iron Workers' Cataract at Wolverhampton. Mr. Cridland showed two typical cases, one in a puddler (reported in 'The Ophthalmoscope,' vol. 13 (1915)), the other in a furnace-man. Mr. St. Clair Roberts showed six cases of chain makers, four of which were typical.

On February 23, 1921, Mr. Parsons, Dr. Morison Legge, and Dr. J. C. Bridge examined cases of cataract in tin-plate rollers at Llanelly. Dr. Healy had collected 121 men, all of whom were examined ophthalmoscopically by Mr. Parsons, and many by Dr. Legge and Dr. Bridge. Nearly all the cases showed some signs of cataract, mostly of the ordinary senile type. Sixteen cases presented the typical appearances of "Glass Workers' Cataract." (These cases form the subject of papers by Messrs. Cridland and Healy in 'The Bristol Journal of Ophthalmology,' vol. 5, pp. 193, 194 (1921)).

There can be no doubt that cataract, indistinguishable from Glass Workers' Cataract, occurs in tin-plate rollermen, chain makers, and puddlers.

Biochemistry of the Lens.

In 1921 the Committee decided to initiate research into the biochemistry of the crystalline lens. Difficulty was experienced in finding a suitable worker to undertake this research. Eventually Miss D. R. Adams of Girton College, Cambridge, was appointed to carry out a research on the metabolism and proteins of the lens under the supervision of Prof. F. G. Hopkins.

Miss Adams' report has been published in the 'Roy. Soc. Proc.,' B, vol. 98, p. 244 (1925), and a résumé of literature on the subject in the 'British Journal of Ophthalmology,' vol. 9, p. 281 (1925). She has proved that "the lens contains an autoxidation system similar to that which Hopkins found in muscles and other tissues. This system in the lens consists of (1) dialysable glutathione, (2) a thermostabile protein residue. These constituents are maintained in constant chemical equilibrium by an oxidation-reduction of an SH ←→ SS type. The peculiar mode of nutrition in the lens prevents any excessive use of oxygen or rapid elimination of waste products. It is therefore conceivable that the autoxidation system is of special economic importance in the respiration of the lens, and that it is not merely secondary in character as it is in other tissues. In favour of this view that it is of vital importance to the normal condition of the lens are the facts that (*a*) in cataractous lenses either or both parts of the autoxidation system may be absent (Goldschmidt), (*b*) the lens in comparison with other tissues has a relatively high glutathione content." Ultra-violet light and heat rays cause a decrease in the glutathione content of the lens *in vitro*, when the latter is protected by a surrounding layer of Ringer's solution. It is as yet unknown whether the secondary (thermostabile) system is affected by the rays *in vivo*. The precipitation of the proteins and the increase of lipoids in the cataractous lens are also inadequately explained. The protein β-krystallin acts alone as a thermostabile residue prepared from the whole lens. Since the other lens proteins are devoid of this power, it appears that β-krystallin is the active constituent of the lens thermostabile residue. The lens is able to oxidise certain organic acids, but its power to do so is decreased by exposure to ultra-violet light.

Since its inception the Committee has lost by death six of its members: Sir William Abney, Sir Clifford Allbutt, Sir William Crookes, Mr. Marcus Gunn, Mr. E. Nettleship, and Dr. A. D. Waller. The present constitution of the Committee is as follows: Sir John Rose Bradford (Chairman), Sir Hugh Anderson, Dr. H. H. Dale, Sir William Hardy, Prof. A. V. Hill, Dr. L E. Hill, and Sir John Parsons.

The investigations carried out by the Committee show that Glass Workers' Cataract is not due to the action of luminous radiation or X-rays; that it is improbable that it is due to direct action of ultra-violet or infra-red radiation upon the lens, though this cannot be definitely disproved until further knowledge of the biochemistry of the lens is available; that it may be due to indirect action on the nutrition of the lens by the deleterious action of infra-red radiation upon the iris and ciliary body.

The introduction of machinery for blowing glass bottles may be expected to reduce the incidence of Glass Workers' Cataract materially. The Committee are of opinion that further research, especially on the biochemistry of the lens, is desirable. They do not, however, feel justified in asking for further financial assistance from the Home Office, but propose that the Medical Research Council be asked to undertake the further pursuit of the investigation.

J. R. BRADFORD,
Chairman.

June, 1928.

The Anaerobic Delayed Heat-Production after a Tetanus.

By W. HARTREE and A. V. HILL, F.R.S.

(Received May 9, 1928.)

(From the Departments of Physiology, Cambridge, and University College, London.)

In a previous paper of this series it was shown by one of us that, in the case of a regular succession of separate twitches, there is no considerable anaerobic delayed heat-production: the only important after-effect of such anaerobic stimulation is a permanent increase in the resting heat-rate. It was pointed out there that this increment, produced by activity, in the basal heat-rate, cannot be regarded as part of the contraction-process itself, though it may easily be misinterpreted; unless the galvanometer-zero and the temperature of the thermopile be very steady, this permanent increase may be mistaken for a long-continued delayed heat associated with contraction and ending in 10 or 15 minutes. The diagram of fig. 1 illustrates this point. At time zero the muscle is stimulated by a short tetanus and the galvanometer deflects, returning to a constant position—but not to its original one—in about 3 minutes. The displacement from the initial position is a measure of the increment in resting heat-rate. This increment must be supposed to occur at, or immediately after, the moment of contraction. There is no obvious reason why it should occur at any other moment. If so, the base-line from which the area of the deflection-time curve must be calculated is A, the continuation backwards of the line representing the final level attained by the galvanometer.

Now let us imagine that owing to galvanometer instability, or to gradual temperature change in the thermopile, there is a slow, small and not quite regular creep of the spot of light on the scale. The only way to proceed is to allow a sufficient time to elapse after stimulating and to join the initial to the final position, so obtaining a base-line (*e.g.*, B in the diagram) from which to measure the area of the deflection-time curve. It is obvious that a greater area will thus be found than if the correct base-line A had been adopted. The case illustrated in the diagram is exaggerated. It will make clear, however, how an error may occur unless extreme stability of galvanometer and thermostat be available. We have found, employing the present apparatus, in all cases, whether of short tetanic stimuli or of a series of single shocks, that with the thermopiles used the galvanometer becomes quite steady again in 3 or 4 minutes : ***there is no long-continued delayed anaerobic heat***, such as was described by ourselves (1) (2) and by Furusawa and Hartree (3). The genuine anaerobic delayed heat is confined to the first 2 or 3 minutes after stimulation.

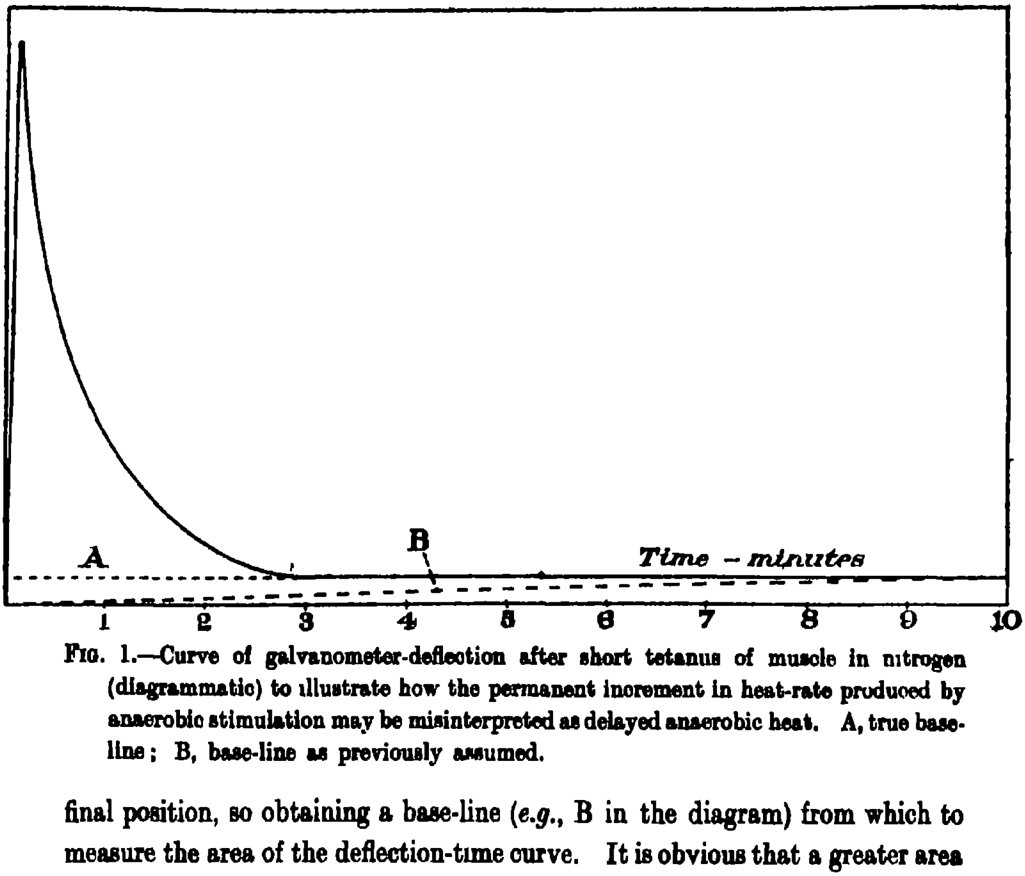

FIG. 1.—Curve of galvanometer-deflection after short tetanus of muscle in nitrogen (diagrammatic) to illustrate how the permanent increment in heat-rate produced by anaerobic stimulation may be misinterpreted as delayed anaerobic heat. A, true base-line ; B, base-line as previously assumed.

In 1923 we (2) concluded that " the effects observed are the resultant of those arising from two separate heat-productions," and fig. 2 of our paper gave diagrammatically the supposed time-course of these two separate factors, (i) starting at a high rate and falling in 2 minutes or so to zero, and (ii) lasting for a long time. Of these (ii) is due solely to the effect, described above, of the permanent increase in resting heat-rate induced by anaerobic stimulation; (i) is genuine, and persists, after a tetanic stimulus, in spite of all efforts to eliminate it.

Experiments have been made in a variety of ways, and with several different thermopiles. It is not easy always to ensure that a muscle under strictly anaerobic conditions will continue to function well enough for a series of tetani to be applied to it. If the condition of the muscle be not good, the experiment must be discarded, since irregularities in the distribution of the heat-production inside the muscle may cause—owing to lag in conduction—apparent effects of delayed liberation or absorption of heat, as pointed out by Furusawa and Hartree (3). One of the best experiments performed, in respect of the continued excellent condition of the muscle throughout, and of the high consistency of the results, was as follows:—

Experiment of 15.10.27.—Two sartorii in nitrogen on quickly acting thermopile; weight 212 mgrs. Two 2-second tetani for test purposes: 195 grms. isometric tension well maintained. Four 2-second tetani with photographic records, practically identical to 12 seconds; mean taken for analysis. Eight "controls" for instantaneous heating. Initial heat in each tetanus, about 0·1 calorie per gram.

The results of the analysis are shown in fig. 2. There is a high rate of heat-production during the development and early stages of the contraction, a

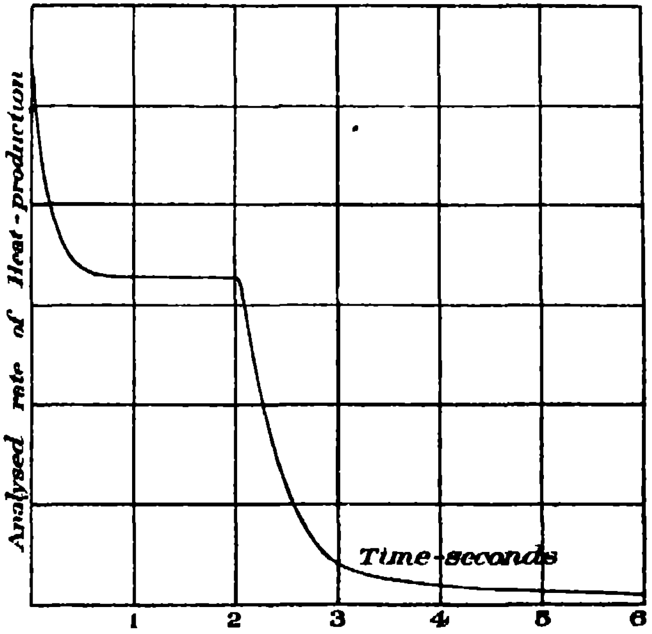

Fig. 2.—Analysis of rate of heat-production during and after a 2-second tetanus in nitrogen. Experiment of 15.10.27 (see text).

constant rate during the maintenance of the contraction, and a rapidly falling rate when stimulation ends. This delayed heat-production persists usually for a minute or two, amounting in some experiments to as much as 46 per cent. of the initial heat, in others to very little. It is greater after longer stimuli; it is absent, or very slow, at low temperatures. It is not to be confused with the heat of relaxation, which is part of the initial process, and is complete in a few tenths of a second at 15° C.

The relaxation heat has been carefully studied at low temperatures, where the anaerobic delayed heat is not a disturbing factor. Experiments to be published later show that the relaxation heat of an isometric contraction at 0° C. is about $0 \cdot 11\ Tl$, where T is the tension developed and l is muscle length. Applying the same formula to the case of 15° C., we may subtract the calculated relaxation heat from the delayed heat shown in fig. 2. It appears that the real delayed heat in 10 seconds after the end of the stimulus was about 15 per cent. of the total initial heat. It is never negative at any stage in the process, though it may, for technical reasons, appear to be in a muscle failing and functioning only in parts: its total amount, to completion, may be anything from 0 or 5 per cent. up to 45 per cent. of the initial heat.

The fact that, in our experience, *there is never any negative heat* affords an important comment on recent observations, in connection with "phosphagen," from Prof. Meyerhof's laboratory in Berlin. It has been demonstrated there that, of the considerable amount of phosphagen "broken down" during a 4-seconds' anaerobic tetanus, an appreciable part (about one-third) is "resynthesised" during the next 20 seconds. The breakdown *in vitro* of purified phosphagen into phosphate and creatine has been shown by Meyerhof and Suranyi (4) to lead to a heat-liberation of about 150 calories per gram of H_3PO_4. If a considerable proportion of the phosphagen "broken down" in anaerobic activity were really "resynthesised" from its constituents during the first 20 seconds after the stimulus, the heat-absorption could not fail to be evident, unless there were some compensating process, *e.g.*, lactic acid or ammonia production, liberating heat. There is no absorption of heat, and, according to Nachmansohn (5) in Meyerhof's laboratory, there is no delayed lactic acid or ammonia production.

It is possible, as was suggested at the end of the third paper of this series, that purified phosphagen has very different thermal characteristics from the substance existing in the living muscle, that, in fact, the heat of formation of the creatine from the precursor actually existing in living muscle is considerably less than from the (possibly simplified) product which has been described by Eggleton and Eggleton. This would reconcile Prof. Meyerhof's observations with the undoubted fact that, at no stage, in or after contraction, does an absorption of heat occur. Another explanation, however, is possible. It may be, as Nachmansohn suggests, that the " breakdown " of phosphagen during activity, and its anaerobic " resynthesis " afterwards, are to be regarded rather as an " unstabilisation," followed by a " restabilisation," of a complex chemical substance, whose real breakdown follows only when mechanical and chemical violence is offered to the living muscle fibres. This suggestion is in keeping with recent experiments of Stella's (6) at University College, in which the interior of a living fatigued muscle has been shown to be in diffusion equilibrium with a phosphate solution containing only about 20 mgrs. per cent. of P: an amount far less than is found, under similar conditions of fatigue, by direct chemical treatment of the muscle (Eggleton and Eggleton (7) (8) (9)). Between these two explanations it is not possible, at present, to decide.

An abstract of the experiments performed is given in the following table. All were at room temperature (15° to 20° C.), and all upon the sartorii of *Rana temporaria* or *R. esculenta*.

Table I.—Delayed Anaerobic Heat (D.A.H.) after a Tetanus.

Experiment of 16.11.28.—Cyanided muscle in N_2. Observations on scale.
0·3 second, 0·5 second, 0·5 second, 0·5 second tetani. Difference between live curves and control disappears in about a minute. D.A.H. *5 per cent.*

Experiment of 17.11.28.—Pure N_2. Observations on scale.
Three 0·4 second tetani. Difference between live curves and control disappears within 2 minutes. D.A.H. *5 per cent.*

Experiment of 14.1.28.—In O_2-HCN. Observations on scale.
1 second tetani. D.A.H. complete in 2 minutes, *13 per cent.*

Experiment of 16.1.28.—In N_2-HCN. Excellent mechanical responses. Observations on scale.
0·75 second tetanus, D.A.H. *15 per cent.*, 7 per cent. before 12 seconds, none after 2 minutes.
1·0 second tetanus. D.A.H. *12½ per cent.*

Experiment of 31.1.28.—Curarised frog. In N_2-HCN. Photographic records.
0·3 and 0·4 second tetani. D.A.H. complete in 50 seconds, *11½ per cent.*

Experiment of 2.2.28.—In N_2-HCN. Photographic records.
0·25 second tetanus, excellent response. D.A.H. *9 per cent.*, 7½ per cent. in 45 seconds.

Experiment of 10.2.28.—In N_2-HCN. Photographic records; detailed analysis; good experiment.
0·15 second tetanus. D.A.H. *23½ per cent.*, 16½ per cent. in 60 seconds.
0·18 second tetanus later. D.A.H. *17 per cent.*

Table I—(continued).

Experiment of 20.2.28.—In N_2-HCN. Photographic records; detailed analysis. Good contractions.

1·13 seconds tetanus. D.A.H., 1st observation, *42 per cent.*, 32 per cent. before 30 seconds. Mean 2nd and 3rd observations, *27 per cent.*, 25 per cent. before 30 seconds.

1·27 seconds tetanus later. D.A.H. *22½ per cent.*, 18 per cent. before 30 seconds.

Experiment of 21.2.28.—In N_2-HCN. Photographic records. Very good and consistent contractions, obvious contracture after each.

2·0 seconds tetanus, 1st observation only. D.A.H. *35 per cent.*, 7 per cent. before 6 seconds, 28 per cent. before 30 seconds.

2·0 seconds tetani, 2nd, 3rd and 4th observations. Mean, D.A.H. *25 per cent.*, 14 per cent. before 30 seconds.

Experiment of 22.2.28.—In N_2-HCN. Photographic records.

1·13 seconds tetanus. D.A.H. *46 per cent.*

1·35 seconds tetanus later. D.A.H. *20 per cent.*, 7 per cent. before 6 seconds, 15½ per cent. before 30 seconds.

Experiment of 24.2.28.—In N_2-HCN. Photographic records. I to VI, 0·1 second tetanus, 0·016 calorie per grm. initial heat. VII and VIII, 1·13 seconds tetanus, 0·091 calorie per grm.

I—D.A.H.	− *2 per cent.*
II—D.A.H.	+ *4 per cent.*
III and IV—D.A.H.	*12½ per cent.*
V and VI—Later	*9 per cent.*
VII—D.A.H.	*30 per cent.*
VIII—D.A.H.	*12 per cent.*

Experiment of 6.3.28.—Photographic records. Detailed analysis shown in fig. 3.

I to III, 0·25 second tetani in O_2, galvanometer returning accurately to zero in 17 minutes. Recovery heat = 1·44 (initial heat).

IV, 0·6 second tetanus in O_2, good return. Recovery heat = 1·32 (initial heat).

V and VI, 0·05 second tetani in O_2, not analysed.

VII and VIII, 0·1 second tetani in O_2, records too small for great accuracy. Recovery heat = 1·02 (initial heat).

XI and XII, 0·25 second in N_2. D.A.H. *11 per cent.*

XIV, 0·6 second in N_2. D.A.H. *23 per cent.*

The last experiment of Table I is illustrated in fig. 3. There are shown (*a*) two typical curves of the delayed anaerobic heat-production for 0·25 and 0·6 second tetanus, (*b*) three curves of the recovery heat-production in oxygen. It is obvious from these that the factor which we have called the "delayed anaerobic heat" is still present in oxygen, complicating the form of the recovery heat-rate curve. This complication does not always, or indeed usually, occur; see, for example, the curves of figs. 1 and 2 of our 1922 paper (1), which are typical. The recovery curves illustrate also a fact which we have several times verified, viz., that (within limits) the rate of recovery heat-production is not only absolutely but relatively greater after a greater initial liberation of energy (see (1), (10)).

An examination of Table I leaves no doubt that after a short tetanus, applied directly to a muscle in nitrogen, there is a delayed production of heat. Every effort has been made to eliminate oxygen, and we have no doubt that the oxygen in the chamber was below 0·02 per cent.;* in most cases the nitrogen

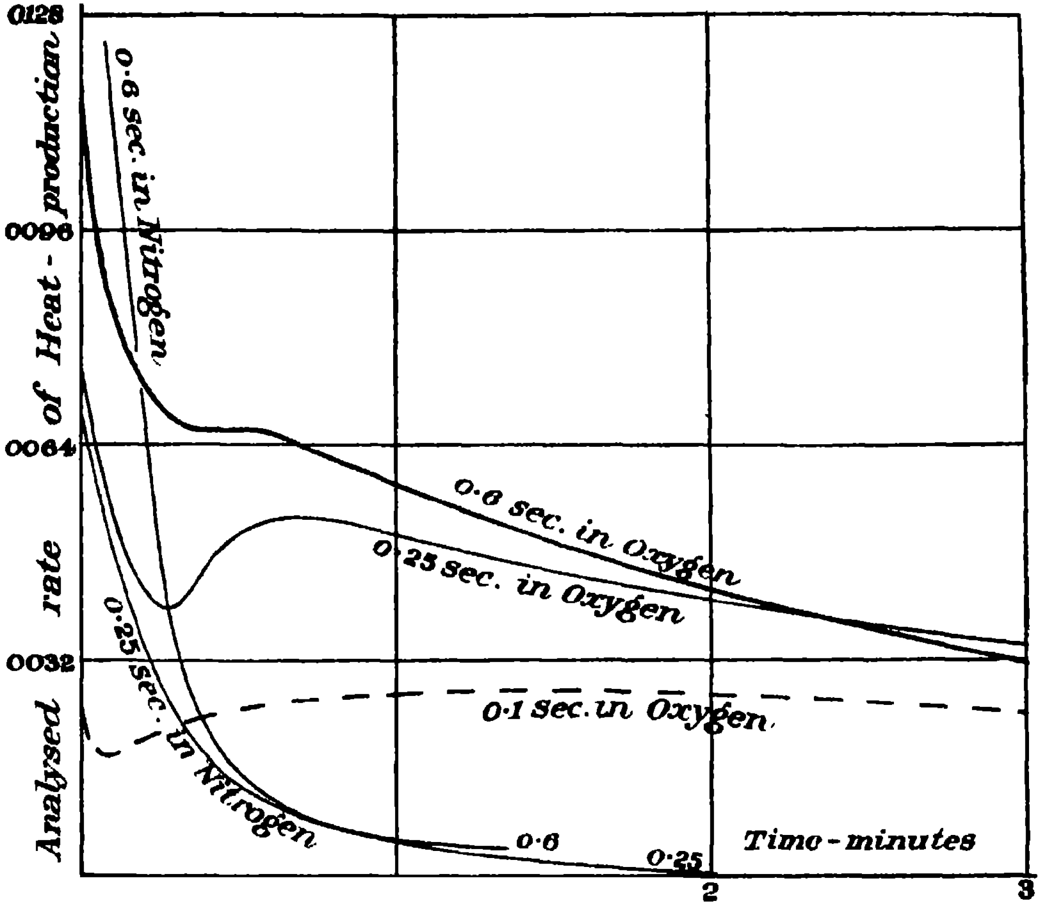

Fig. 3.—Analysis of rate of heat-production after a short tetanic stimulus, in oxygen and in nitrogen. Experiment of 6.3.28, (see text and Table I). The rate of heat-production is measured in terms of a unit which is equal, in each observation, to the initial heat per second.

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* In this connection we have never been able to verify, even under the most stringent anaerobic conditions, a statement by Furusawa and Hartree (3) to the effect that when "purified nitrogen was passed through the muscle chamber for an hour or so . . . the muscle became inexcitable." Our muscles have often given an excellent response after hours in the purest nitrogen, even when dosed with cyanide vapour. We believe that the inexcitability described by Furusawa and Hartree must have been due to some anæsthetic substance present in their nitrogen, which had been vigorously shaken with alkaline pyrogallol. Ours, as described in the first paper of this series, had been made with alkaline sodium hydrosulphite ($Na_2S_2O_4$) and passed through four wash-bottles before it reached the muscle chamber.

further contained cyanide vapour, in amount sufficient to abolish the recovery process even in pure oxygen. The amount of this delayed anaerobic heat is variable. It appears, in general, to be greater after long tetani than after short ones—which is in keeping with the fact that it is absent in the case of a series of single twitches: it is usually greater in the earlier contractions of an experiment: in Table I it varies from 5 per cent. to 46 per cent. of the initial heat, having usually a value in the neighbourhood of 15 per cent. It starts at a high rate, and falls rapidly to zero in a minute or two; it is practically absent at 0° C., or so slow as to be impossible to detect with any certainty (which is a further proof, if one be required, that it is not due to a physical error of any kind). The facts are clear; the explanation is obscure.

The muscles were excited directly by thick silver-wire electrodes in good contact with their ends. The stimuli were just maximal, not supermaximal; the tetanus from a Harvard coil was employed. It has been suggested to us, in view of the possiblity of over-stimulating some of the muscle fibres when direct stimuli are employed, that indirect excitation would be more conclusive. If a muscle nerve-preparation were available which would meet the rigorous demands of the myothermic method, indirect stimulation would certainly be preferable. The gastrocnemius-sciatic preparation, however, is useless for the purpose: in dealing with such small differences as are here involved, an accurate "control" is essential, in the form of a uniform warming of the dead muscle. This requires a muscle of uniform cross-section, with fibres all working in the same way, and preferably a very thin one, to avoid the effects of lag in conduction from parts contracting to different degrees. We do not believe that results of any value would be obtained in this connection with a muscle much thicker, or less uniform, than the sartorius of the English frog. It is, of course, possible to prepare the nerve to the sartorius. There are two reasons against this:—

(*a*) Strictly anaerobic conditions would certainly tend gradually to destroy the power of the nerve fibres to conduct an impulse; and

(*b*) This preparation is so delicate that one could never be sure that certain of the fibres of the nerve had not been injured.

In either case some of the fibres of the muscle would respond to the stimulus applied to the nerve, others would not. The distribution of non-contracting fibres would probably not be a random one; discrete masses of the muscle would fail to respond, and we should get temperature differences inside the tissue itself, giving the apparent effect of delayed production or absorption of heat The simple experiment described by Furusawa and Hartree (3, p 204) of scorching the outside surface of the muscle with a hot wire, treatment which results in an apparent large negative heat soon after the contraction, shows the danger of a non-uniform response It appeared to us to be impossible to ensure that a muscle stimulated through its nerve, after a long period in pure nitrogen, should contract uniformly throughout If such surety be absent, it is useless to perform the experiment, the records could indeed be made and analysed, but whatever result was obtained would be suspect it might be attributed, if desired, to a non uniform contraction of the muscle

The dangers of the myothermic method, improperly used, are great, and recent experience shows what extraordinary results it may yield in the hands of uncritical people, long acquaintance with its pitfalls only emphasises its limitations The sole way, in our opinion, to hope to secure a uniform contraction of a muscle under strictly anaerobic conditions, is to stimulate it directly In that case the possibility of over stimulating some of the fibres—those in immediate contact with the electrodes—cannot be avoided

In 1926 Embden Hirsch Kauffmann and Deuticke (11) published an account of experiments purporting to show that considerable quantities of lactic acid may be produced by a stimulated muscle after contraction and relaxation are complete This effect was proved by Meyerhof and Lohmann (12) to be due to the excessive stimuli they employed excited through its nerve or directly by a more moderate current a muscle liberates lactic acid only during its contraction The matter was further studied by Suranyi (13), who showed that too strong a stimulus caused (i) an excessive production of lactic acid per unit of tension maintained in a tetanus and (ii) a contracture afterwards Furusawa and Hartree (3), in confirmation of these results, obtained a large after production of heat in consequence of an over powerful stimulus There is no doubt of the danger of excessive direct stimulation and according to recent results of Nachmansohn (5) it is difficult to avoid this danger so long as direct tetanic stimuli are employed

Thus one possible explanation of the delayed anaerobic heat, observed in the present experiments, is that it is due to the excessive stimulation of those fibres of the muscle which lie directly on the electrodes, where the density of the exciting current is greatest In several of the experiments recorded in Table I an undoubted contracture occurred after normal relaxation This explanation would be in keeping with the great variability of the effect observed it is difficult indeed to see how its possibility can be eliminated It is clear from the experiments with single twitches that a considerable production of heat is not a *necessary* sequel to an anaerobic contraction The over stimulation theory of its appearance after a tetanus has much to recommend it

There is however, an alternative explanation which should be mentioned. The experiments on phosphagen made in Prof Meyerhof's laboratory, and already referred to, show that for half a minute or so after a tetanus of several seconds there are chemical processes at work, involving changes in the phosphate containing complexes of the muscle It may not be correct to describe these as the actual resynthesis of " phosphagen " from creatine and phosphate, which is an endothermic reaction, but undoubtedly some chemical changes are proceeding It seems possible that the anaerobic delayed heat may be a sign of these processes

Of the two explanations considered we are more inclined to the former The recovery heat production is a very regularly occurring phenomenon , so is the relaxation heat observed at a low temperature , so is the increment in resting heat rate produced by anaerobic stimulation , and it seems to us probable that if the anaerobic delayed heat discussed above were due to normal processes occurring inside the muscle it would appear in twitches and in tetani alike, and not to the very variable degree illustrated in Table I

Summary

1 The anaerobic delayed heat production after a tetanus has been re-examined, with stringent precautions to avoid the possibility of oxidation

2 Its supposed long continued part is due to a misinterpretation of the phenomenon described in a previous paper, viz , the permanent increment in resting heat rate produced by anaerobic stimulation

3 Its earlier part is a genuine occurrence and is complete within a minute or two It is very variable in amount ranging from 5 to 46 per cent of the initial heat It starts at a high rate and falls rapidly to a low value, being complete in a minute or two

4 Its explanation is obscure it may be due to phosphate changes known to occur after contraction and relaxation are complete , more probably it is to be attributed to the over stimulation of some of the fibres of the muscle, a procedure known from the work of Meyerhof and his colleagues to produce a delayed formation of lactic acid

5 There is no sign of an endothermic process occurring at any stage in, or after contraction The partial restoration of phosphagen after an anaerobic tetanus as described by Meyerhof and his collaborators, must be regarded,

therefore, either as a "restabilisation," rather than as a "resynthesis," or as showing that purified phosphagen possesses very different thermal properties from the precursor of phosphate and creatine in the living muscle.

6. We have been unable to verify a statement by Furusawa and Hartree that in purified nitrogen a muscle becomes gradually inexcitable, as a nerve is known to do. A muscle may give an excellent response after hours in the purest nitrogen, to which cyanide vapour has been added.

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The Equation of Motion of a Runner, exerting a Maximal Effort.

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(Communicated by Prof. A. V. Hill, F.R.S.—Received June 4, 1928.)

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Furusawa, Hill and Parkinson (1) have shown that the acceleration of a runner and the maximum speed he can obtain depend upon two factors, (*a*) the maximum force he can exert in propelling himself, and (*b*) the frictional resistance of his muscles. They have embodied these factors in a mathematical equation, which they have tested by experiment. The experimental results fit the equation very satisfactorily and allow the values of the constants to be determined.

The maximum velocity attained depends upon a balance between the propelling force, which is constant up to the onset of fatigue, and the internal resistance, which increases as the speed rises until it balances the propelling force. In spite of the accuracy with which experiment has been found to verify the equation, this internal resistance has to some degree a hypothetical existence. The object of the present paper is to give it greater reality by showing how an actual external resistance can be added on to it and produces exactly the calculated effect.

In the equation developed by Furusawa, Hill and Parkinson, if M be the mass of the subject, and $f\mathrm{M}g$ be the maximum propelling force he can exert, and if the internal frictional resistance at any speed be taken as M/a times that speed, then the maximum velocity has the value of fga. If now to the internal frictional resistance of the muscles we add an external force, R, the maximum speed will be further reduced in proportion, and instead of being fga it will be $(fga - \mathrm{R}a/\mathrm{M})$.

In their paper Furusawa, Hill and Parkinson suggested that experiments should be made on running uphill, where the external resistance would be the component of the weight of the body parallel to the surface of the hill. In the present paper another type of external resistance has been employed, namely, that exerted by a cord wound around a capstan, braked by the arrangement shown in the illustration (p. 220).

Methods.

The apparatus used for the electrical timing of the runners was practically the same as that used by Hill and his collaborators. In fact, parts are identical, since we are indebted to them for the gift of the recording coils. In our experiments a Moll moving-coil galvanometer (Kipp en Zoonen) was used. This instrument has a complete period of 1·3 seconds. The lag of the recording apparatus (1, p. 37) was 0·04 second. A spectrograph slit was used to narrow the beam of light and thus to sharpen the peaks of the deflections. A pendulum with a period of about 1 second was used as a time marker in most of the experiments. The period was determined at frequent intervals by timing several hundred complete vibrations. In later experiments a clock with a long wire pendulum was substituted.

Other features of the recording apparatus were practically identical with those described by Hill. The coils of insulated wire were usually placed at the following distances from the start, 1, 3, 5, 7, 9, 12, 15, 21, 25, 30, 35, 37·5, 40, 42·5, 45 and 50 yards. The coils were concentrated at the start to give the initial acceleration, and between 30 and 50 yards to determine more accurately the maximum speed, which was found, by experience, to be attained by our subjects within that range. The start was automatically recorded by the closing of an electric switch which was included in the pistol.

To obtain the constant external resistance a metal drum, braked by a measurable force, was constructed. This drum with its horizontal shaft revolved on ball bearings. A strong light cord was wound on the drum and the free end attached to a broad belt fastened about the runner's waist. The unbraked drum exerted no measurable resistance, except at the moment of the start. The slight pull at the start unwound the cord much more rapidly than the runner could take up the slack. The drum was braked by means of a linen band which encircled a narrower drum fastened to the common shaft. The ends of the band were fastened to two spring-balances, which were attached to a framework above the shaft. The balances could be raised or lowered by means of a screw and the friction on the drum thus increased or decreased. The pointers on the balances registered the friction as soon as the runner started and this remained constant throughout the run.

The first few experiments were conducted on an outdoor track, the remainder were carried out on an inside track where a 60-yard "straight-away" was available (see figure). The constants in the equation of motion were determined for several of the members of the University of Toronto track team, but it was found advisable to use one subject (C.H.B.) for most of the experiments in which the effect of external resistance was studied. This subject could reproduce his maximum speed in different runs on the same day with great consistency (Table II). As a routine procedure, the subject ran first without an external resistance, then made two or three runs with different external resistances, and a final run without resistance. An appropriate interval between successive runs was always allowed, to permit complete recovery from the previous run. The final run without external resistance was always recorded, in order to make certain that fatigue due to previous efforts had not reduced the maximum speed of the subject. It was considered advisable not to add very large external resistances which might produce a change in the stride of the runner. The resistances we have used produced no perceptible change in the stride or style of running.

a, Recording coil; *b*, Spring balances; *c*, Cord from capstan to runner's waist; *d*, Linen friction band.

The photographic records obtained of the deflections of the galvanometer were fastened on a wooden block. Vertical lines were drawn through the peaks of the waves, and through the same side of every alternate gap which was produced in the record by the pendulum. The distances were measured by a steel ruler graduated in 1/64-inch divisions, and these measurements were checked by a vernier microscope reading to 1/100 cm. This value was then inserted in the equation, and if found satisfactory was adopted. This was almost always the case, but sometimes a very slightly different value was more appropriate. In determining Ra/M, if the external resistance be expressed in pounds weight, this value must be multiplied by g (10·73 in yard/second units) to obtain R in Hill's equation. For C.H.B., whose weight was 170 pounds, M is 170. Ra/M varies from 0·10 to 0·56, according to the change in R.

Experimental Results.

In calculating the change in maximum speed of a subject which should be produced by a certain external resistance, it is necessary to know the value of the constant a. In Table I fga, a and f are given for several subjects, the last three of whom were used in the experiments in which the effect of external resistance was studied.

Table I.

Subject.	fga.	a.	f.	M.
J.H.R.	10·60	1·355	0·73	150
E.C.M.	10·70	1·40	0·71	160
E.W.McH.	7·96	1·033	0·71	130
H.P.	9·20	1·20	0·71	160
W.S.	7·90	1·00	0·74	130
C.H.B.	8·87	1·325	0·62	170

The constancy of the maximum speed attained in the two control runs on the same day by the subjects used is satisfactory. The values for these control runs and the number of sprints made by the subjects with external resistance are shown in Table II.

Table II.—Maximum Speed without a Resistance.

Subject.	Before exercise.	Amount of exercise.	After exercise.
C.H.B.	8·86	3 runs	8·87
C.H.B.	8·87	2 runs	8·88
C.H.B.	9·12	2 runs	9·12
C.H.B.	8·93	3 runs	9·14
C.H.B.	8·93	2 runs	8·98
C.H.B.	8·89	3 runs	8·89
C.H.B.	8·74	3 runs	8·74
W.S.	8·06	2 runs	8·04
W.S.	7·87	3 runs	7·95
W.S.	7·88	3 runs	7·86
H.P.	9·20	2 runs	9·20

In the seven experiments in which subject C.H.B. was used, the maximum speed attained in the preliminary run agrees well with that in the final, with one exception. On one occasion the maximum speed for the preliminary run was 8·93, and for the final 9·14 yards per second. As the maximum speeds in these control runs do not show satisfactory agreement, the results are not used in Table IV. This is the only trial which is eliminated. It is interesting that the subject stated before making the final run in this experiment that he felt tired and was doubtful of his ability to equal his preliminary performance. The average difference between the preliminary and final maximum speed for the remaining 10 experiments is 0·019 yard per second.

In Table III examples of the figures obtained in two experiments with and without external resistance are given. The subjects used in the experiments always attained their maximum speed by the time they reached the 45-yard mark. The results for the four runs given in Table III show the speed between

Table III.—Results with and without Resistance.

Subject.	Resistance (in grams)	Distance (in yards).	Time from preceding coil (in seconds).	Average speed (yards per second).	Maximum speed (yards per second).
C.H.B.	0	21	—	—	8·88
		30	1·060	8·5	
		35	0·564	8·86	
		40	0·564	8·86	
		45	0·563	8·88	
		50	0566	8·83	
C H.B.	650	21	—	—	8·75
		30	1·069	8·42	
		35	0·571	8·75	
		40	0·571	8·75	
		45	0·572	8 74	
		50	0·599	8·34	
C.H.B.	0	30	—	—	8·89
		35	0 582	8·5	
		37·5	0·289	8·6	
		40	0·281	8·89	
		42 5	0·298	8·4	
		45	0 300	8 3	
		50	0·599	8 3	
C.H.B.	2250	30	—	—	8·47
		35	0·633	7·9	
		37·5	0·310	8 0	
		40	0 300	8·3	
		42 5	0 295	8·47	
		45	0·295	8·47	
		50	0·637	7 9	

the coils placed at the 30- and 50-yard marks. The sudden decrease in speed sometimes observed after the 45-yard coil is reached is due to the fact that there is a sharp turn in the indoor track just beyond the 50-yard coil.

Table IV.—Decrease in Maximum Speed due to a Constant Resistance.

Experiment No.	Subject.	Resistance (in grams).	$R\alpha/M$.	Observed maximum speed without a resistance ($f/g\alpha$).	Maximum speed with a resistance.	
					Calculated.	Observed.
1	C.H.B.	550	0·10	8·88	8·77	8·76
2	C.H.B.	650	0·12	8·88	8·76	8·75
3	W.S.	650	0·12	8·04	7·92	7·86
4	W.S.	700	0·13	8·06	7·93	7·90
5	W.S.	750	0·14	7·87	7·73	7·74
6	C.H.B.	750	0·14	8·86	8·72	8·71
7	C.H.B.	750	0·14	8·86	8·72	8·71
8	H.P.	750	0·15	9·20	9·05	9·05
9	C.H.B.	850	0·16	8·74	8·58	8·58
10	C.H.B.	850	0·16	8·74	8·58	8·57
11	W.S.	875	0·16	8·06	7·90	7·93
12	C.H.B.	1000	0·18	8·74	8·56	8·55
13	H.P.	1025	0·18	9·20	9·02	9·02
14	C.H.B.	1075	0·20	8·86	8·66	8·67
15	C.H.B.	1125	0·21	8·88	8·67	8·68
16	W.S.	1125	0·21	7·59	7·38	7·36
17	W.S.	2175	0·40	7·81	7·41	7·40
18	C.H.B.	2250	0·41	8·89	8·48	8·47
19	W.S.	2400	0·45	7·81	7·36	7·35
20	C.H.B.	2500	0·46	8·95	8·49	8·47
21	C.H.B.	2600	0·48	8·89	8·41	8·39
22	C.H.B.	3050	0·56	8·95	8·39	8·37

The results of the experiments in which an external resistance was added are collected in Table IV. The average difference between the calculated and observed maximum speeds for the first 16 experiments is approximately 0·015 yard per second. That is, the average difference between the maximum speed actually observed and that calculated from the equation of Furusawa, Hill and Parkinson is not greater than the average difference in the maximum speeds of the two control sprints made on the same day.

This is a satisfactory demonstration that the internal viscous resistance of the muscles is not just a hypothesis invented to make the equation fit the observations, but is real, in the sense that it has identically the same effect as an external added resistance.

Hill (2) has recently calculated the effect of a head wind on the speed of a runner. The effect of a head wind would be the same as the external resistance in our experiments, except that the wind pressure is distributed all over the projected area instead of being located in the middle of the runner's body, as is the tension of the cord. The results reported in this paper confirm Hill's calculations and show that if 1 kilogram be the added effect of any particular head wind, it will produce a certain calculable diminution of the maximum speed of the runner. As most of our experiments were conducted on an indoor track, changes in air resistance, due to wind, did not occur. Hill's calculations show, however, that a decreased speed, such as that produced in our experiments by the added resistance, involved a smaller air resistance. This factor was not taken into account in calculating the maximum speeds shown in Table IV.

Using data which are available in Hill's paper (2), calculations show that the change in air resistance due to the decreased speed is negligible in the first 16 experiments in Table IV. In experiment 15, for example, the calculated value for the maximum speed with a resistance would be 0·01 yard per second greater if the change in air resistance had been considered. In experiments 17 to 22 the effect of the decreased air resistance is more important. In experiment 18 the calculated maximum speed would be approximately 0·03, and in experiment 22 approximately 0·04, yard per second greater if the change in air resistance, as well as the resistance afforded by the drum, had been considered.

These calculations show that there is not quite such good agreement between the calculated and observed values of the maximum speeds, in the experiments with large external resistances, as the figures in Table IV indicate. The observed maximum speeds are consistently slightly lower than those calculated. It is possible that the expenditure of energy in starting the drum and in acceleration when large resistances are used prevents the runner from attaining quite as great a maximum speed as he would if the same resistance had been more gradually applied.

When the effect of the decreased air resistance is considered in calculating the maximum speed in experiments 17 to 22, the average difference between the observed and calculated maximum speeds in the 22 experiments is approximately 0·025 yard per second.

Summary.

Experiments in which external resistances of varying magnitude have been applied to a runner show that the maximum speed of the subject is decreased by the amount calculated from the equation of Furusawa, Hill and Parkinson. This is a satisfactory demonstration that the internal resistance of the muscles

is real, in the sense that it has identically the same effect as an external added resistance.

These experiments were initiated as a result of a discussion of recent work on the dynamics of sprint running with our colleague Dr. P. J. Maloney. Our sincere thanks are due to Prof. A. V. Hill and Prof. John Satterley for their helpful interest in the research. It is a pleasure to acknowledge the skilful technical assistance of Mr. Wm. Parkinson.

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The Dynamics of Bicycle Pedalling.

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According to recent developments in the theory of muscular action, the average external force exerted during a muscular movement, carried out with maximal effort, may be regarded as equal to a constant theoretical force diminished by an amount proportional to the speed of movement. As a deduction from this, the relation between certain quantities involved in a specified type of muscular exercise can be expressed in the form of a mathematical equation. The equation can then be tested by experiment. Certain kinds of human limb movements have already been subjected to this form of analysis (5), (6), (7), (8). In the present paper is described a similar investigation of the movements of pedalling a bicycle.

Consider the case of a subject pedalling a bicycle against a constant resistance. The resistance might be due to a hill of constant slope, or, in the case of laboratory experiments with a bicycle ergometer, to the friction of a band applied to the wheel. Let P be the maximum force (averaged over the whole range of foot movement) that can be exerted by the leg at right angles to the pedal crank when the rate of pedalling is such that one foot movement (*i.e.*, half a complete revolution of the crank) is completed in t seconds. Then according to the theory, the relation between P and t should be capable of expression in the form,

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* Working for the Industrial Fatigue Research Board of the Medical Research Council.

$$P = P_0 (1 - k/t),$$

where P_0 and k are constants. P_0 represents the maximum force that could be exerted at right angles to the pedal crank, and would be attained only if the movement could take place infinitely slowly; while k represents the shortest time in which the movement could be completed, and would be attained only if no external work were done. The constants have a theoretical meaning only, and cannot be measured directly. If the theory holds, they should be characteristic for a given subject in a given bodily condition.

Let α be a constant, such that αR is the force that must be exerted at the pedal to overcome R at the rim of the wheel. The value of α depends on the dimensions of the bicycle. Now when the bicycle is started from rest, the force P is equal to αR, plus the rate of change of momentum of the moving parts, multiplied by a suitable constant. As the rate of pedalling increases, the internal frictional resistance in the muscles $P_0 k/t$ increases, and the external force given by $P_0 (1 - k/t)$ decreases, until it becomes equal to αR. Then no further increase of speed can occur. Thus a maximum speed is reached.

If t be the time of one foot movement when the maximum speed is attained, then

$$\alpha R = P_0 (1 - k/t).$$

This gives a relation between the "load" R and the maximum speed $1/t$ that can be reached. With a bicycle ergometer it is possible to make observations of the maximum speed of pedalling that can be attained when pedalling against a given load. By plotting the observations so obtained, a graph can be drawn showing the relation between load and maximum speed. If the above equation holds, then the graph obtained should be a straight line cutting the axes at a speed $1/k$ and a load P_0 respectively. Thus by experiment it is possible to ascertain whether the above relation holds, and, if it does, to determine the value of the constants P_0 and k for any subject.

Details of Experiments.

A friction bicycle ergometer as designed by Martin (1) was used. A modified arrangement was adopted for applying the tension to the ends of the friction band. The cord leading from the end of the band coming from the underside of the wheel was led over a pulley mounted on ball-bearings fixed in front of the bicycle, and tension was applied by hanging weights on the free end of the cord. The other end of the band was sewn round a piece of aluminium tubing, from the ends of which cords passed on either side of the uprights of the bicycle, supporting saddle and handle-bars, to a spring-balance fixed horizontally in front of the bicycle. When the spring-balance was to be read by the bicyclist instead of by a second experimenter, it was fixed to the upright supporting the handle-bars and so lay horizontally between the cyclist's legs. When the bicycle is being pedalled the difference between the weight hung on the cord and the tension recorded by the spring-balance gives the force exerted at the rim of the wheel. The use of a weight instead of a spring-balance on the "positive" side has the advantage that stretching of the cord or band during an experiment will not cause any permanent change in the force at the rim. The spring-balance read by 0·1 kgm. up to 10 kgms.

The turns of the wheel were recorded by a signal magnet, writing on a smoked drum and excited when a contact was made, once in each revolution of the wheel. A second signal magnet wrote on the drum, the circuit exciting which was made and broken by a metronome. The metronome made contact on alternate beats, at intervals of about 1 second; the interval between contacts was determined by calibration with a stop-watch. The speed of the drum was arranged so that 1 second was represented by about 2·7 cm. From the record so obtained the speed of pedalling was determined.

The gearing of the bicycle was 3 pedal revolutions to 8 wheel revolutions. The radius of the wheel was 25 cm. The length of the pedal crank was 18 cm. Hence the component of the force exerted by the foot in a direction perpendicular to the crank was equal to 3·7 times the force exerted at the rim of the wheel. The pedals of the bicycle were fitted with toe-clips so that the subject could press the pedals forward as well as downward.

In performing an experiment a series of readings was taken, starting with the lighter loads, increasing to the heavier loads, and then repeating some of the lighter ones. For each reading the subject started with one pedal ready to be pushed downwards at 45° from the top position. The wheel could then be started with a jerk. The subject pedalled as fast as possible for about 10 seconds, the maximum speed being attained within this time. Between readings the subject rested in a chair. In order to ensure constancy in the type of movement, it is important that there should be no sideway movements of the body or rising from the saddle. The pressure exerted by the legs is counterbalanced by the weight of the body, and by the arms pulling upward on the handle-bars.

The range of loads that can be used in this experiment is limited at both ends of the scale. With a very light load the feet can move so quickly that they tend to fly off the pedals and the subject cannot induce himself to pedal as fast as his muscles would let him. The lightest load possible for men was found to be about 5½ kgms. at the rim of the wheel, and for women about 3 kgms. With the heaviest loads a point is reached at which the subject is unable to move the pedals when they are in the least advantageous parts of the movement. This load was reached for women at about 10 to 12 kgms., differing, of course, for different individuals. For men it was not reached within the range of loads used. In practice the range is more restricted, because with heavier loads, approaching the heaviest that can be moved, the pedalling becomes very uneven. It is to be noted that all the loads used were heavier than those generally employed in experiments with the ergometer bicycle.

Results.

It was found that a maximum speed of pedalling was reached after about 4 seconds from the start. After about 7 to 10 seconds an appreciable decrease of velocity occurred, owing to the onset of fatigue. In Table I are shown the

Table I.

Subject.	Weight.	Height.						
	kgm.	cm.	Load	3·1	4·5	5·6	6 4	
D.H. (1)	53·5	175	Time of leg movement, seconds	0 232	0·282	0 366	0 433	→
				0 250	0·281	0 374	0 440	←
			Load	3·1	5·0	6·2	7·8	9 2
S.D. (2)	53·5	160	Time of leg movement, seconds	0·205	0·245	0·280	0·375	0 461→
				0 203	0·260	0·281	0·369	0·460←
			Load	7·0	9·4	10·3	11·8	
H.D.D. (3)	84·5	176	Time of leg movement, seconds	0·240	0·280	0·305	0·335	→
				0·235		0·305		←
			Load	4 5	5·6	6·8	9·4	10·4
S.M. (4)	71·0	170	Time of leg move-movement, seconds	0 244	0·265	0·305	0·440	0·559→
				0·236	0·261	0·295	0·427	0·520←

The load is given in kilograms at the rim of the wheel.
Subjects (1), (2) and (4) are women.

results obtained on one man and three women. In these experiments, after the greatest load had been reached, the observations were repeated, using the same loads in descending order. In Table I the second observation with a given load is put below the first, in order to show how accurately the result for the maximum speed is reproduced on the second occasion. The results do not consistently show the effects of either fatigue or practice, except in the case of the subject S.M., who gave a slightly higher result for the maximum speed in all the repeated observations.

From the results of these experiments graphs were plotted, the ordinate representing the force at the rim of the wheel, and the abscissa the number of half-revolutions per second. Fig. 1 shows the graphs obtained from three of the experiments: that of the fourth is so close to the middle one of the other three that it is omitted. Similar experiments performed on the subjects D.H. and S.D. on other occasions gave lines substantially the same as those shown for these subjects in fig. 1.

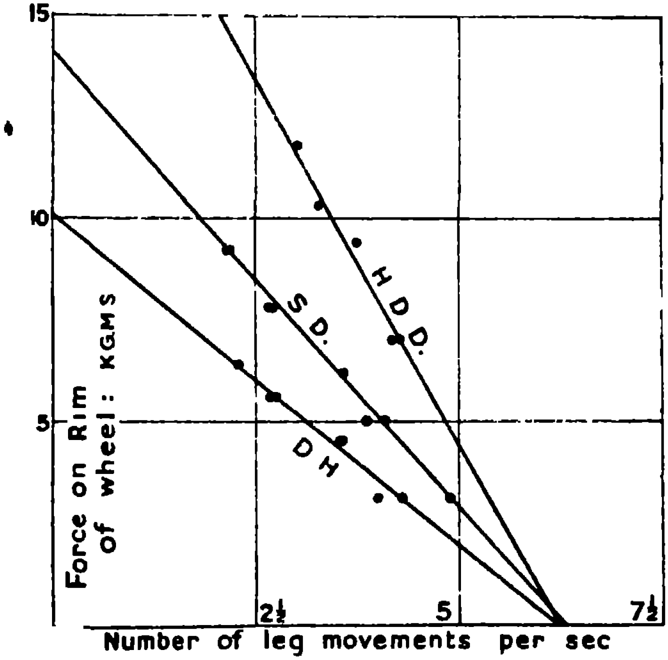

FIG. 1.—Relation between force overcome at rim of wheel and number of half-revolutions per second at maximum speed for three of the subjects of Table I (D.H. and S.D. are women).

Measurements were made also on two other men and one other woman. The results of all the experiments, that is, on three men and four women, are shown in fig. 2. The graph for each individual was first drawn and the values of the constants found. Then for each observation values of the ratio of the force to the theoretical maximum force, and of the speed to the theoretical maximum speed, could be calculated. These values are plotted together in fig. 2. It is seen that the points are distributed about a straight line.

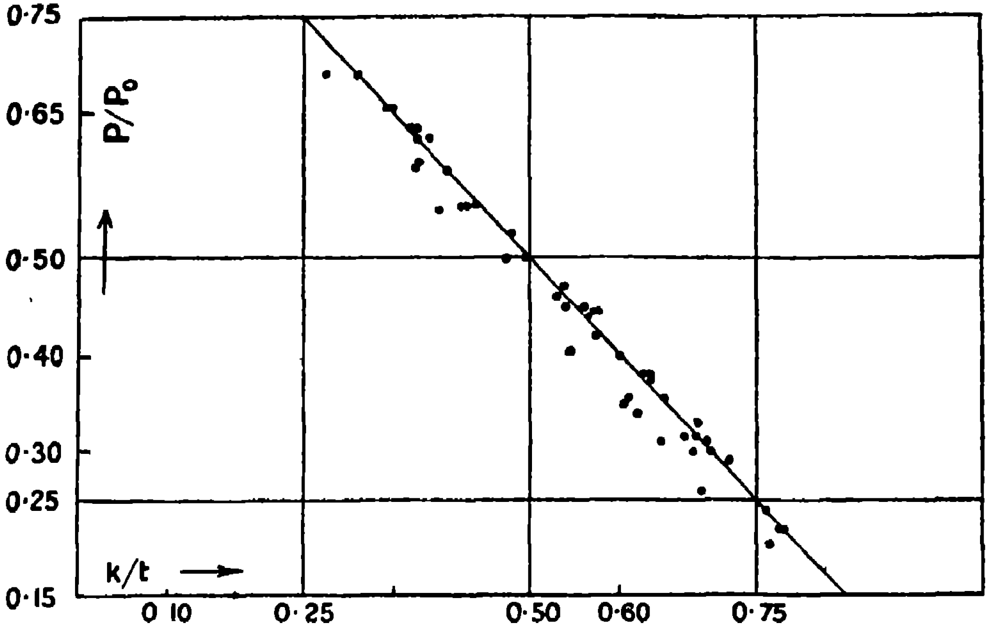

FIG. 2.—Relation between P/P_0 and k/t for seven subjects, three men and four women. To obtain the values P/P_0 and k/t the values of P, the load, and $1/t$, the maximum speed, found for each subject were first plotted. From the graphs obtained, P_0, the maximum force, and $1/k$, the maximum speed, were found for each subject. Then P/P_0 and k/t were calculated for each experimental value of P and $1/t$ and plotted, the purpose being to make the observations on all subjects directly comparable in a single diagram.

From the graphs of fig. 1 it is seen that k, the theoretical minimum time, is not very different for different subjects. The values lie between 0·159 and 0·162 second. The experiments on the other subjects gave values of the same order. On the other hand, the value of P_0, the theoretical maximum force, shows wide differences, the most marked being those between the values given by men and by women. For the women, the values of P_0 lay between 37 kgms. and 56 kgms.; for the men, they lay between 75 kgms. and 85 kgms.

From the results it is interesting to calculate (1) the theoretical maximum force expressed as a fraction f of the body weight; (2) the theoretical maximum work W_0 done in one foot movement. For the four subjects of Table I, these values are as follows :—

Table II.

	D.H.	S.D.	H.D.D.	S.M.
P_0, kgm.	37·5	52	83	55·5
f	0·70	0·97	0·97	0·78
W_0, kgms.-metres	21·1	29·4	46·8	31·4

It is important to note that the force P measured in the experiments is the component, perpendicular to the crank, of the force exerted by the foot averaged over the whole range of movement. Hence, P_0 gives the maximum force perpendicular to the crank, averaged over the whole movement.

Since the way in which muscles come into play is different at different stages of the movement, and since the pressure on the pedals is not adjusted to be at right angles to the crank at all points, the effective turning force varies at different stages of the movement. If the muscles are always used in the same way, then the average effective turning force will be a constant proportion of the average force actually exerted. These considerations serve to explain why the value of f is lower than would be expected. The maximum force averaged over the whole range of movement is less than the maximum force that can be exerted in the most advantageous positions of the pedals, that is, when the crank is horizontal. In the most advantageous position of the pedal, the bicyclist can exert a force greater than his own weight; the maximum force averaged over the whole movement is less than his weight

The force P_0 has a certain practical meaning. Since it is shown that the internal resistance, due to the "viscosity" of the muscles, has the same effect as an externally applied force, the maximum force P_0 represents the total effective turning force exerted in a maximum effort, and is used partly in overcoming the external resistance and partly in overcoming the internal resistance due to muscle viscosity.

The constants P_0 and k, found from the experiments, are constants for a given individual, under given bodily conditions for the prescribed movement. Values of the constants as determined depend not only on the qualities of the muscle involved, but also on the range of movement permitted, and on the way in which the contraction of the muscles used is related to the movement of the extremity of the limb and to the effective force exerted by the limb. If, for example, the movement employed were of only half the full extent of which the muscles were capable, the value of k (which may be regarded as the

" theoretical minimum time " for an unloaded movement) would be half that corresponding to the full movement. The fact that the k found here is less than that given by Lupton (6) is presumably due to this cause.

Discussion.

In their paper on the "Dynamics of 'Sprint' Running," Furusawa, Hill and Parkinson (8) showed that the assumption (*a*) of an internal resistance proportional to the speed and (*b*) of a constant propelling force would explain the acceleration of a runner and his attainment of a maximum speed. Their equations predicted that a constant external resistance applied to a runner, *e.g.*, by a wind, would reduce his maximum speed in proportion to the resistance.

The experiment has actually been made by Best (9) and the prediction verified. The experiments recorded here are fundamentally of the same nature as those of Best; the maximum speed is found to decrease as a linear function of the load. The linear relation existing in man between force exerted and speed of movement was deduced originally from experiments with an inertia ergometer (5), (6). It has been confirmed (*a*) by the existence of an optimum speed (7), (*b*) by experiments on the acceleration of a " sprinter " (8), (*c*) in the experiments by Best on the effect of applying a load to a runner, and (*d*) by the present observations. As a quantitative expression of the relation between force exerted and speed attained, it may be regarded as satisfactorily established in the case of muscular movement in man.

One conclusion of importance can be drawn from the existence of a linear relation between speed and load. It was shown theoretically by A. V. Hill (5), and confirmed experimentally by Lupton (7), that such a relation must lead to the existence of an optimum speed of movement, at which the mechanical efficiency is greatest. The same considerations apply exactly in the present case, and experiments to be described in a later paper confirm the existence of an optimum speed, and show that in pedalling on a road, efficiency demands the choice of a gear-ratio allowing a rate of leg movement not too far from the optimum.

Summary.

1. Recent studies of the maximum speed of human muscular movement have indicated that an external applied resistance should cause a proportional diminution in the speed. In the present experiments the maximum speed of pedalling a bicycle ergometer has been determined as a function of the load applied to the wheel.

2. The relation between maximum speed and load is linear, the speed decreasing as the load increases according to the equation $P = P_0 (1 - k/t)$; here P is the force exerted at right angles to the pedal crank, P_0 the "theoretical maximum force" attained only at zero speed, k a "viscosity" factor, the "theoretical minimum time," and t the actual time of a single leg movement.

3. The value of k, the "viscosity" factor, is much the same in all the subjects examined; P_0, the "strength" factor, varies widely from one to another.

4. It is deduced that bicycle pedalling, as other forms of movement involving the overcoming of an external resistance, will show an optimum speed at which the mechanical efficiency is highest.

My thanks are due to Prof. A. V. Hill for his interest and help during the investigations, to Mr. Downing and Mr. J. L. Parkinson for the construction of the ergometer, and to all those who acted as subjects for the experiments.

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The Factors determining the Maximum Work and the Mechanical Efficiency of Muscle.

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In order, in a prolonged contraction, to obtain the maximum work from a muscle, the load must be so adjusted that at every stage the muscle is just, and only just, able to overcome it; and the speed of shortening must be as low as possible. Levin and Wyman (1), in their work on the "viscosity" of muscles, employed an ergometer which, allowing the muscle to shorten at any desired speed, measured the maximum work which it was capable of performing at that speed. Their instrument, which records a tension-length curve on a fixed smoked surface, is very accurate and convenient to use, and it is theoretically inconceivable that greater work—at a given constant speed—could be recorded by any other means. The only way to increase the work is to decrease the speed, in order to reduce the energy wasted in overcoming the internal resistance of the muscle.

From the point of view of the mechanical efficiency of muscle (*i.e.*, ratio of work done to total energy liberated) prolonged contractions are to be avoided, since they require large amounts of energy to be liberated in maintaining them (7). The matter has been discussed by one of us and his colleagues in several places (2), (3), (4), (5), p. 32, (6), pp. 48 and 81. It is clear that for a high efficiency the contraction must be of comparatively short duration: there is indeed, for human muscles, an optimum duration at which the efficiency is greatest. We were led therefore to a consideration of the maximum work obtainable in the response to a short stimulus, and to an experimental determination of the maximum mechanical efficiency of the frog's isolated muscle. The matter is much more complicated than is the simple case of a prolonged contraction, considered by Levin and Wyman, and it has required the examination of the effects of varying several different factors.

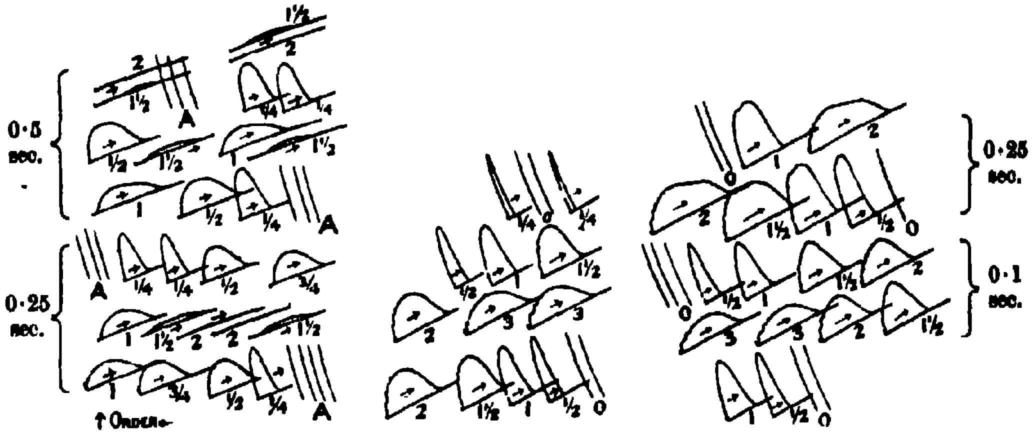
The ergometer employed was the identical instrument described by Levin and Wyman ((1), p. 222). Its records (see figs. 1 and 4 below) were made by a lever writing on a smoked glass surface, and were reproduced on photographic paper as contact prints. The moving member was released by an electromagnet, the current in which was broken at any desired moment by an arm on the revolving contact breaker (8), which determined the duration of stimulus. Its speed was adjusted by altering the setting of the needle-valve, which regulated the rate of flow of oil. The work done by the muscle was calculated in gram-centimetres from the area of the tension-length curve. The muscles used were in all cases a pair of frog's sartorii, from *Rana esculenta* or *R. temporaria*. They were placed upon a thermopile in a muscle chamber of the type described by Hartree and Hill (9). The initial heat-production was recorded in the usual manner on a galvanometer scale. The temperature of the muscle was that of the Dewar flask in which the chamber stood.

In all that follows it should be understood that reference is to contractions of limited duration, and that the initial phase only of contraction (*i.e.*, not including recovery) is considered, unless the contrary be stated.

(1) *The Variation of Work with Speed of Shortening.*

If the speed of shortening be too low, little work will be done, owing to the fact that relaxation sets in, and the tension of the muscle disappears, before the process of shortening is complete. If the speed be too high, again little external work will be done, but in this case because of the internal resistance (" viscosity ") of the muscle, which increases with the rate of shortening. At intermediate speeds the work is greater, and attains a maximum at one particular speed.

These factors are well illustrated by the records of fig. 1 and the curves of fig. 2. In the set of records denoted 16.5.27, in fig. 1, a pair of frog's sartorius muscles was employed at 0° C., and subjected to a tetanus (*a*) of ¼ second (lower half) and (*b*) of ½ second (upper half). Starting at the lower right-hand corner, the muscle was given 4 isometric stimuli of ¼ second. It was next allowed to shorten at a speed denoted by ¼ (which represents the adjustment of the needle valve regulating the speed), doing work equivalent to the area of the first tall thin curve. The arrow on each curve denotes the direction of shortening It will be noticed that the movement was so slow that the muscle had relaxed before shortening was complete. The speed was then increased to the value denoted by ½, and another curve recorded, the height being less (owing to muscle " viscosity " hindering the development of tension at the higher speed), but the width greater (owing to a greater amount of shortening before relaxation set in). The speed was then adjusted successively to the values denoted by ¾, 1, 1½, 2, 2, 1½, 1, ¾, ½, ¼, ¼; and finally three isometric records were taken. The results are very regular. The greatest



16.5.27
0·25 and 0·5 sec. 0° C.

21.5.27
0·1 sec 15° C.

15.6.27
0·1 and 0·25 sec. 14·5° C.

Fig. 1.—Effect of speed of shortening on the work done in a contraction of limited duration. Tension-length records, made by the Levin-Wyman ergometer, in three experiments. Actual size. Length, 20° from horizontal; tension, 20° from vertical. Arrows show the direction of shortening. Numbers refer to the speed of shortening given by the setting of the needle-valve of the ergometer. A or O = isometric. (For details, see text and fig. 2.)

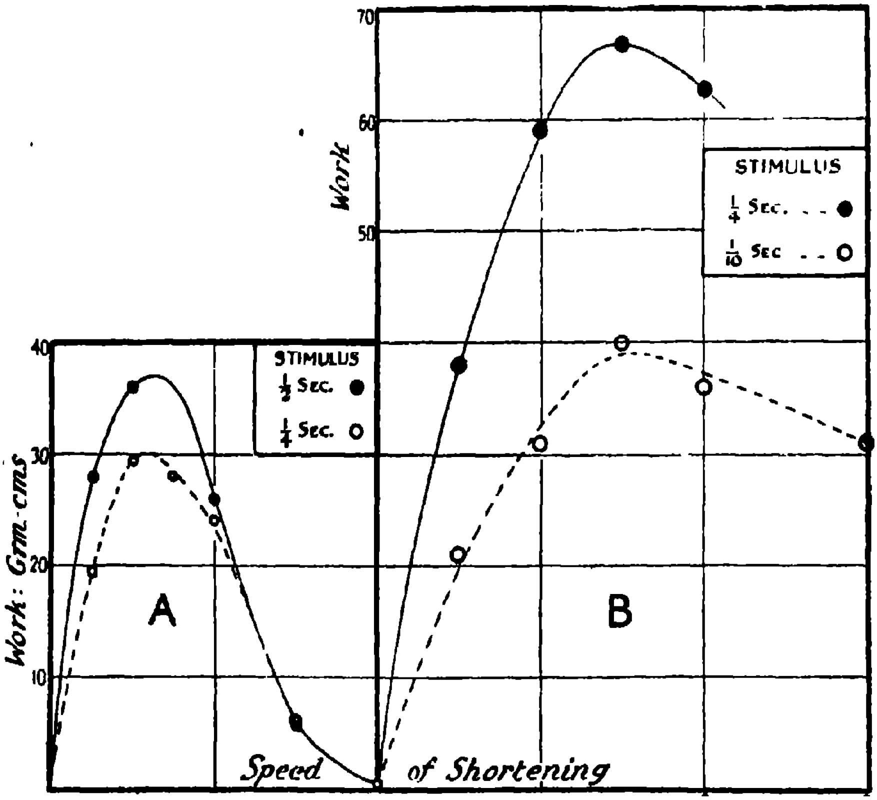


Fig. 2.—Effect of speed on the work done in a contraction of limited duration. Graphical representation of the results of two experiments of fig. 1. A = experiment of 16.5.27 at 0° C; B = experiment of 15.6.27 at 14·5° C.

force is developed at zero speed (isometric) and the maximum tension steadily falls as the speed increases, until finally practically no force is developed, and no work done, at the highest speed, denoted by 2.

The experiment was then repeated with a tetanus lasting ½ second, in the order reading from right to left and proceeding upwards. The same identical phenomena appear. The work done, as calculated from the areas of the curves, is expressed in fig. 2, A, as a function of the speed. It is seen to rise from zero at zero speed (isometric), to pass through a maximum, and to fall to zero again at the higher speeds.

For the records denoted 21.5.27 in fig. 1 a pair of sartorii was stimulated for 0·1 second at 15° C. Starting again from the bottom right-hand corner there are, in order, contractions at speeds of shortening denoted by 0, ½, 1, 1½, 2, 3, 3, 2, 1½, 1, ½, ¼, ¼, and 0. The higher temperature, having diminished the duration of contraction, has made the slower shortenings (*e.g.*, ¼ and ½) less effective: relaxation sets in far too early to allow a considerable amount of work to be done at such speeds. On the other hand, the higher temperature has diminished the "viscosity" of the muscle, and so given a greater effectiveness to higher speeds of shortening. Consequently at speed 2, where practically no work was done in the experiment at 0° C., the work at 15° C. is near its maximum: even at speed 3 the work has not fallen considerably. It would require very high speeds, beyond the effective range of our instrument, to reproduce at 15° C. the whole curve of fig. 2, A. The results of this experiment are as follows ·—

Speed	0	¼	½	1	1½	2	3
Work..........	0	9	19	31½	43½	43	41

For the records denoted 15.6.27 in fig. 1 a pair of sartorii at 14·5° C. was stimulated for 0·1 second (lower group) and for 0·25 second (upper group), the speeds of shortening being as shown, and the direction, as usual, from left to right. The onset of relaxation is easily seen in the curves for the longer stimulus. The results of this experiment are shown in fig. 2, B. The work done is greater than at the lower temperature, but it is obvious that exactly the same type of relation exists between work and speed, the speed necessary to obtain the maximum work being about 2½ times as great at 14·5° C. as at 0° C.

(2) *The Variation of Work with Duration of Stimulus.*

It was shown by Fick many years ago ((10), p. 61) that a muscle allowed to shorten in such a way that, at every stage, it is just able to overcome the external resistance to which it is opposed, will perform an amount of work equal to its theoretical maximum, viz., to the area of its tension-length curve. We know now, particularly from the work of Levin and Wyman (1), that the attainment of such maximum work in a prolonged tetanus requires a very low speed of shortening; in fact the lower the speed, the greater the work (neglecting the possible incidence of fatigue). In this paper we are not concerned with prolonged contractions, only with those of limited duration, and we have seen above that in such contractions, owing to the onset of relaxation, there is a definite speed at which the work is greatest. The curves, moreover, of fig. 2 show, so far as they go, that for a given speed of shortening the work increases as the duration of the stimulus increases. We will now examine, in greater detail, the relation between work and duration of stimulus.

In Table I are given the results of 11 experiments, in each of which the relation between work done and speed of shortening was determined, as

Table I.—Relation of Maximum Work, and corresponding Speed, to Duration of Stimulus. (Speed is given in settings of the needle-valve on the piston.)

Number	1			2		3		4	
Temp., ° C.	14½			15		15		15	
Duration (seconds)	0·03	0·10	0·25	0·10	0·25	0·06	0·20	0·03	0·10
Max. work (grm. cms.)	19	48½	67	29	52	17½	42	17	33
Speed	1·6	1·6	1·5	1·7	1·8	1·5	1·5	2·0	2·0

Number	5		6		7					
Temp., ° C.	15		15		0			4 (later)		
Duration (seconds)	0·03	0·10	0·03	0·10	0·06	0·25	0·50	0·06	0·25	0·50
Max. work (grm. cms.)	28	42	37	63	19	30	37	14	27	29
Speed	2·0	2·0	1·4	1·4	0·65	0·7	0·7	0·75	0·8	0·65

Number	8		9		10		11	
Temp., ° C.	0		0		0		0	
Duration (seconds)	0·25	0·50	0·50	1·0	0·50	1·0	0·25	0·5
Max. work (grm. cms.)	23	26	38½	59	46	57	30	37
Speed	0·65	0·6	0·6	0·4	0·65	0·45	0·65	0·65

described above, for at least two durations of stimulus. The curves representing this relation were similar, in every case, to those of fig. 2, and it is unnecessary to record their characteristics in detail. We shall refer only to the *greatest* work, and the speed at which that work was attained. The work was plotted as a function of the speed; the maximum work, and the speed of shortening corresponding to it, were then read off, and are given in Table I for each duration of stimulus.

From Table I we see that, as would be expected, the maximum work increases with duration of stimulus, but that over the comparatively narrow range of durations dealt with the optimum speed (*i.e.*, the speed giving maximum work) is practically independent of the duration. The first of these conclusions is always true: the second is for the narrow range of stimulus-duration corresponding to a twitch-like form of contraction. It is well known that a short tetanus produces a much stronger, but not a much longer response, than a single shock. If the stimulus be prolonged there is no doubt that the optimum speed, for maximum work, is reduced; in a very long contraction the optimum speed is very low indeed, as Levin and Wyman showed. We see the beginning of this effect in experiments 9 and 10 of Table I. For the shorter durations, however, up to 0·25 second at 15° C., and up to 0·5 second (probably even to 0·75 second) at 0° C., the chief effect of prolonging the tetanus is to increase the strength, rather than the length of the response, and within this range we find that the optimum speed, for maximum work, is independent of the duration of stimulus. Indeed, from the table, it is obvious that the optimum speed, for a given temperature, is practically the same for different muscles, as well as independent of the (short) duration of the stimulus. This is a great advantage to the experimenter, for it means that the optimum speed and maximum work can be realised at once, practically without trial, by a standard setting of the ergometer.

The manner in which, for a given speed of shortening (corresponding approximately to maximum work), the work varies with the duration of stimulus, is shown by the experiments of Table II.

Table II.—Variation of Work with Duration of Stimulus: Constant Speed.

A.—*Temperature*, 0° C. Work in gram-centimetres. Duration in seconds.

Duration.	0·06	0 25	0·5	0·75	1·0
Work, experiment 1	19	29	41	[54]	61
" " 2	20	33	40	[55]	63
" " 3	18	27	37	46	55
" " 4	18	28½	39	47½	54½
" " 5	13	22	[32]	[40]	49
Work, mean	18	28	38	49	57

Quantities in square brackets were interpolated between others at slightly different durations.

B.—*Temperature*, 15° C.

Duration.	0·03	0·1	0·2	0·3
Work, experiment 1	16	31½	41	53
" " 2	18	33	39½	—
Work, mean	17	32	40	53

The results of Table II are shown in fig. 3. The work, for a given speed of shortening, increases with duration of stimulus much more rapidly at a high

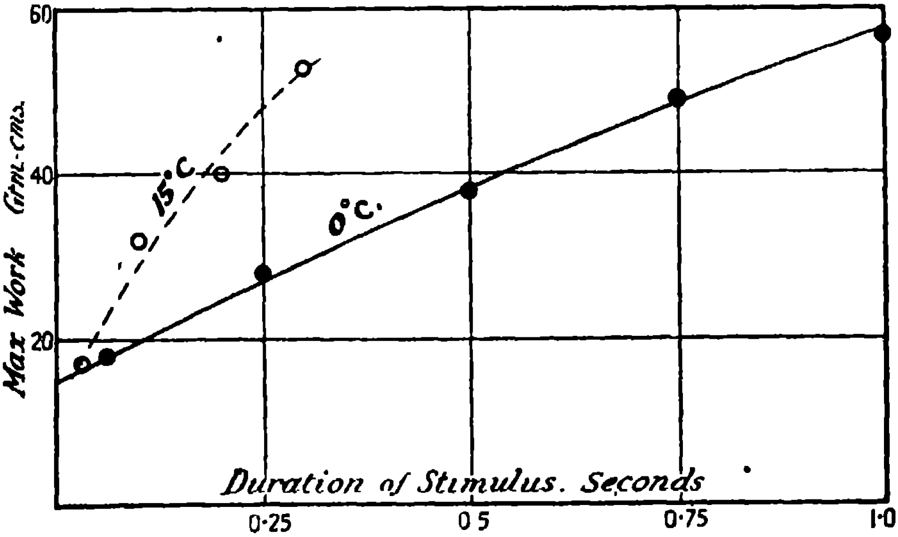


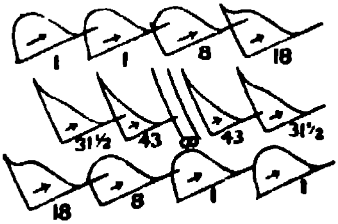
Fig. 3.—Relation between maximum work and duration of stimulus, in a contraction of limited duration.

temperature than at a low one, in the same way as does any other characteristic of the mechanical response, *e.g.*, the isometric tension. The maximum work is obviously, for many purposes, the best measure of the mechanical response, and its relation to duration of stimulus is shown in fig. 3.

(3) *The Effect, on the Work, of varying the Moment of Release.*

If the ergometer be timed to start directly the stimulus is applied, the tension of the muscle will not have reached its full value, indeed in the early stages of contraction it may be exerting very little force: consequently the work done may not be as great as if the muscle be held fast initially and allowed to shorten only after its tension is more fully developed. It is not possible, *a priori*, to calculate how large this effect of a later release may be; experiments therefore have been made to determine its magnitude.

In the experiment denoted 13.5.27 in fig. 4, two sartorii at 0° C. were stimulated for half a second, and the ergometer was released at various intervals (represented by 1, 8, 18, 31½ and 43 divisions of the revolving contact breaker) after the beginning of the stimulus; these correspond to times of 0·016, 0·125, 0·285, 0·50 and 0·68 seconds respectively. The speed of shortening was very slightly less than the optimum. Starting from the right-hand bottom corner the records are given in order, shortening being from left to right as shown by the arrows. In the curves denoted 1 the movement clearly began very slightly before the tension of the muscle was manifest. In the record denoted 8 the tension had attained about half of its full isometric value (see the lines noted ∞) before shortening began. In record 18, 80 per cent. of the full tension was attained before release; in record 31½, 95 per



13.5.27.
0° C. 0·5 sec.

FIG. 4.—Effect of a delayed release on the work done at a given speed of shortening in a contraction of limited duration. Tension-length records, actual size, as fig. 1. Numbers refer to the interval between beginning of stimulus and moment of release, expressed in divisions of the contact-breaker (63 = 1 second). (See text and fig. 5.)

cent.; in record 43, 100 per cent. Two isometric stimuli were then applied, and the series repeated in reverse order. The results were as follow :—

Moment of release (seconds)	0·016	0·125	0·285	0·50	0·68	∞
Mean work (grm. cms.)	44½	49	44	35	24½	0

It is clear that a definite, though small, advantage is gained by a later release. A number of such experiments have been performed, with results given in the following table.

Table III.—Effect of Delayed Release on the Work Performed. (Times of release are given in divisions of the contact-breaker, of which 63 = 1 second.)

Experiment 1.—0° C. ¾ second tetanus. Optimum speed.

Time of release	1½	8	18	31½	43
Work (grm. cms.)	38	39	36	29	22

Experiment 2.—6° C. ¼ tetanus. Nearly optimum speed.

Time of release	1½	7½	15 7	23	31
Work (grm. cms.)	29½	29	20	13	7½

Experiments 3, 4, 5.—13° C. 0·1 second tetanus.

Time of release	0	1	2	3	4	5	6	7	8	9	11	12	13	14	15	17
Work, experiment 3	40	40	43	39	38	—	33	—	26	—	15	—	—	5½	—	—
" " 4	—	25	27	26	—	23	—	17	—	11	—	3	—	—	0	—
" " 5	—	34	40	41	—	34	—	28	—	20	10	—	3	—	—	0

Experiment 6.—14° C. 0·03 second tetanus.

Time of release	1	2	3	4	6
Work	21	19	16	14	7

Experiment 7.—15° C. 0·1 second tetanus.

Time of release	0	2	6·3	11
Work	44½	44	34	17

The results of experiments 3, 4, and 5 are exhibited in fig. 5. The relation between work and time of release was plotted for each of them, and a curve having the mean height of the three was calculated. The optimum interval between beginning of stimulus and moment of release is quite short: it depends upon the temperature (*i.e.*, on the quickness of muscular response) and upon the duration of stimulus (for a single twitch it would be very short). At 13° C. with 0·1 second tetanus it is about 0·04 second. In attempting to attain the maximum work and efficiency, the improvement resulting from a slight delay in release, though small, cannot be neglected.

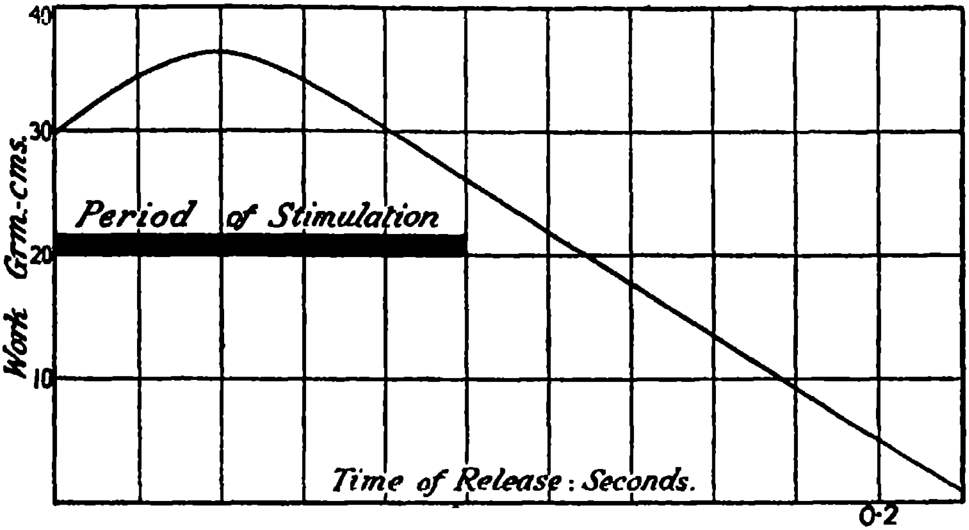


FIG. 5.—Effect of a delayed release on the work done at a given speed of shortening in a contraction of limited duration. Mean of three experiments at 13° C., see text.

(4) *The Maximum Efficiency of Muscle.*

We have described above the conditions which determine the maximum work in a short contraction: the attainment of this maximum requires a certain optimum speed of shortening, and a certain small delay in the release of the ergometer. If the heat-production be measured at the same time as the work, we can, by adding these together, obtain the total energy; thus the quantity $\frac{\text{work}}{\text{total energy}}$ which we may call the "efficiency" of the initial process, can be calculated. It has recently been shown by one of us (11) that the ratio of total to initial heat in an isometric contraction is about 2·07, and by the other of us (12) that this ratio is unaffected, substituting the word energy for heat, by a considerable amount of work done in the contraction. If, therefore, we can determine the maximum "efficiency" of the initial process, then by the simple expedient of dividing it by 2·07 we can calculate the maximum "efficiency" of the whole muscular cycle. The "efficiency" of the isolated muscle has been determined before, but never with such a satisfactory arrangement for obtaining maximum work as the ergometer employed in the present experiments.

Strictly speaking, the maximum work and the maximum efficiency may not occur under precisely the same conditions. The total energy is known to vary with the work (Fenn (13) (14)), and if the variation of total energy were considerable, and if the conditions determining the position of its maximum were considerably different from those determining the maximum work, then the ratio $\frac{\text{work}}{\text{total energy}}$ might attain its greatest value at a different position from either numerator or denominator. Actually, however, the variation of the total energy is only a comparatively small fraction of its whole amount, and, moreover, the total energy is practically stationary within the region of the maximum work: in fact, therefore, the maximum efficiency is found so close to the maximum work that in practice it is sufficient to determine the maximum work and the simultaneous value of the total energy, and then dividing one by the other the maximum efficiency is obtained.

In many cases, in the following experiments, the speed and moment of release were varied, simultaneous observations of work and heat being made, and the maximum efficiency found after plotting the data as functions of the quantity varied. In other cases long experience of the conditions under which maximum work is obtained was assumed to warrant standard settings of speed and moment of release. The experiments were made with various durations of stimulus and at various temperatures.

The heat-production was measured in the manner adopted now for several years in the Cambridge Laboratory. The heat was calculated for the whole muscle (since the whole muscle does work) after the usual calibration, multiplying by the factor $\frac{\text{weight of whole muscle}}{\text{weight between calibrating electrodes}}$.

The results of 22 separate experiments, made on 21 pairs of sartorius muscles, at intervals between March 1 and June 27, 1927, are given in Table IV. The contractions were all of unlimited extent (the muscle shortening as far as it was able), with a short interval between beginning of stimulus and release, and at or very near the optimum speed of shortening; the muscles were in good condition throughout.

Table IV.—The Efficiency of the Initial Process of Contraction. (Note: the efficiencies are given as a per cent.)

Experiments 1 *to* 10, *at* 0° C.

Duration (seconds).	0·06	0·25	0·5	0·6	0·7	0·75	1·0
Efficiency, experiment 1	22	23½	24	—	—	—	24½
,, ,, 2	27½	27½	26½	—	26	—	26½
,, ,, 3	25½	26	27½	—	—	28½	29
,, ,, 4	24	24	25	—	—	25	25½
,, ,, 5	20	21	—	23	—	—	24½
,, ,, 6	—	—	25	—	—	—	26½
,, ,, 7	—	—	32	—	—	—	27
,, ,, 8	—	20	27½	—	—	—	—
,, ,, 9	—	—	—	—	—	—	27
,, ,, 10	31½	33½	31½	—	—	—	—
Mean of 1 to 5	24	24½	26	—	—	—	26

Mean of all 26 per cent.

Experiments 11 *and* 12, *at* 4° *C.*; *experiment* 13 *at* 6° *C.*

Duration (seconds).	0 06	0 25	0·5
Efficiency, experiment 11	—	27	28½
,, ,, 12	23	25½	21½
,, ,, 13	—	—	23½

Mean of all 25 per cent.

Experiments 14 *and* 15 *at* 13° *C.*; 16 *at* 14½° *C.*; 17, 18, 19, 20, 21, 22 *at* 15° *C.*

Duration (seconds).	0 03	0·06	0·10	0 20	0·25	0·3
Efficiency, experiment 14	—	—	28	—	—	—
,, ,, 15	—	—	27½	—	—	—
,, ,, 16	24	—	26½	—	27	—
,, ,, 17	—	—	—	27½	—	—
,, ,, 18	27	—	24	—	—	—
,, ,, 19	26	—	29½	27	—	28
,, ,, 20	—	—	30	—	—	—
,, ,, 21	—	—	24	—	23	—
,, ,, 22	—	23	—	26	—	—

Mean of all 26 per cent.

The results of Table IV are seen to be very consistent. At 0° C. there appears to be a very slight tendency for the maximum efficiency to be higher at the longer durations, but no such tendency is visible elsewhere. There seems to be no effect of temperature on the maximum efficiency. The average value for the 56 observations recorded in the table is 26 per cent.; the average deviation of a single reading from the mean is just over 2 per cent. Such observations as these involve a great many measurements; they are necessarily subject to experimental errors of several kinds, and in addition there are inevitable variations in the tissues employed. Considering all this the consistency with which the 56 independent values given in Table IV group themselves round the mean of 26 per cent. is a sign of the real and essential constancy of the maximum efficiency of the isolated muscle. This maximum is unaffected by temperature, and—within the limits of a "short" contraction as defined on p. 239 above—by the duration of the stimulus.

The value of 26 per cent. for the maximum efficiency of the initial process is not far greater than the 20 per cent. found on the average for the frog by Fenn ((13), p. 194) for an isotonic contraction after-loaded with a suitable weight, and only slightly greater than the 23 per cent. found by him for a contraction against an inertia lever. For the toad, Fenn's average values are, isotonic contraction 21 per cent., inertia lever 29 per cent. A discussion of the meaning of this low efficiency of the frog was given by Fenn: here we would emphasise again the astonishingly small proportion of the energy liberated by the frog's muscle which can be transformed into external mechanical work.

It is difficult, or impossible, to imagine any means of allowing the muscle to do work, more efficient than that employed in the present experiments. Apart from avoiding or diminishing the effects of the internal "viscosity" of the muscle, theory cannot suggest any way of further improving the method of work-collection. Yet, taking the initial process alone, only 26 per cent. of the energy of the glycogen-lactic acid reaction can be turned into external mechanical work, while taking the whole cycle, initial and recovery, only about 13 per cent. of the total energy of oxidation can, as a maximum, be so transformed. In man we have strong evidence of a 25 per cent. efficiency over the whole process. There are many likenesses between the muscles of man and frog; it may be consoling, however, to the former to know that his efficiency can attain values twice as high as that of the latter. The divergence is really larger than it seems; for human muscles, owing to their attachments in the body, and to other factors, never work under such ideal conditions as those provided by the Levin-Wyman ergometer. It would be interesting to apply methods similar to those used in the present investigation to the muscles of other cold-blooded animals, to find whether these also have so low an efficiency.

The efficiency of human muscular movement, as was shown by one of us (2) and by Lupton (3), is determined by a balance between two factors, (i) the viscosity of the muscle, tending to diminish W (the work done) below its theoretical maximum W_0, by an amount proportional to the speed of shortening, and (ii) the energy required to maintain the contraction long enough to allow a sufficient amount of work to be done.

In previous papers the expression adopted for factor (i) was $W = W_0(1 - k/t)$, where k represents the viscosity and t the duration of the stimulus: while for factor (ii) the initial energy H for a contraction W_0 was taken to be

$$H = aW_0(1 + bt),$$

where a is a quantity representing the energy required *to set up* a contraction of unit strength, and b is a quantity such that energy ab is liberated, per second of stimulus, in *maintaining* it. The efficiency is then

$$E = \frac{1 - k/t}{a(1 + bt)}.$$

There is an error in this statement of the case, arising from the fact that the duration of the contraction, which determines the maximum work, is greater than the duration of the stimulus; obviously $t = 0$ for a single shock, but considerable work can be done owing to the fact that a contraction of finite duration (say c seconds) ensues.

In previous discussions of the problem this fact was neglected; the times involved were sufficiently long to ensure that no serious error resulted. In the present case, however, the error would be serious, and instead of t in the expression $W = W_0(1 - k/t)$ we must write $(t + c)$, so that

$$W = W_0(1 - k/(t + c)), \tag{I}$$

where c is a time representing the duration of the contraction in excess of the duration of the stimulus. The second factor is unaffected by this consideration, in fig. 4 of our paper (7) H/Tl was shown to be approximately a linear function of the duration of the stimulus. It is correct, therefore, to write

$$H = aW_0(1 + bt). \tag{II}$$

The efficiency then is

$$E = \frac{1 - k/(t + c)}{a(1 + bt)}. \tag{III}$$

Now it can be shown by differentiation with respect to t that the expression (III) for E has a maximum at a value $t = t_0$ given by

$$t_0 = k(1 - c/k + \sqrt{1 - c/k + 1/bk}) \tag{IV}$$

and that this maximum value is

$$E_{\text{max.}} = \frac{1}{abk\,(1+\sqrt{1-c/k+1/bk}\,)^2}. \tag{V}$$

Now from (IV), if $\theta_0 = (t_0 + c)$ be the duration of the contraction giving the highest efficiency,

$$\theta_0 = k\,(1+\sqrt{1-c/k+1/bk}). \tag{VI}$$

From this the simplest expression for the maximum efficiency can be derived,

$$E_{\text{max.}} = k/ab\,\theta_0^2. \tag{VII}$$

But we found in Table IV above, for the case of frog's muscle, that the efficiency with very short stimuli is the same as with slightly greater ones. Hence the optimum value of t, $t = t_0$, is zero, or approximately zero: the highest efficiency is attained with very short stimuli. Thus the maximum efficiency is given by the expression, substituting $t = 0$ in (III),

$$E_{\text{max.}} = (1 - k/c)/a.$$

In frog's muscle a is approximately unity, as was shown by one of us (15). Putting $E_{\text{max.}} = 0 \cdot 26$, as found in Table IV, we finally obtain

$$1 - k/c = 0 \cdot 26 \qquad k/c = 0 \cdot 74.$$

Thus the maximum efficiency, in a frog's muscle, is determined by the ratio of the viscosity to the time occupied in a single twitch. The maximum efficiency of the frog occurs for very short contractions, which is natural, since the frog is built for rapid jumps and not for weight-lifting. Moreover, we know that, when the temperature is raised, the viscosity diminishes in like measure as the time occupied in a twitch. Thus k/c remains constant, and the maximum efficiency is independent of the temperature.

Now it is not inevitable that the efficiency should be greatest for short times of contraction. In man, very rapid movements are far from being the most efficient: the greatest efficiency is attained, as Lupton (4) and others have shown, with comparatively slow movements. In fig. 1 of Lupton's paper, which deals with the case of stair-climbing, the most efficient duration of a single movement of the leg is shown to be about 1·3 seconds. Inserting this value in equation (VII) we find

$$E_{\text{max.}} = k/1 \cdot 69\, ab.$$

Now k is known, from Lupton's experiments on human arm muscles (3) carrying out a maximal degree of shortening, to be 0·26. For movements involving less than the maximal amplitude of shortening, the value of k (which represents the minimum time of an unloaded movement) would be smaller. Let us assume, for the process of climbing a stair, that $k = 0 \cdot 20$. There is no means of ascertaining a and b directly for the case of man. The total energy, however, in the whole process (including recovery) can be measured by respiratory methods, and Lupton ((4), p. 345) determined the energy used per second in maintaining a maximal pull of the arm muscles. His subject W.B., in maintaining a pull capable of theoretical maximum work $W_0 = 10 \cdot 96$ kilogram-metres, used $5 \cdot 32$ kilogram-metres of energy per second; in the symbols employed here, regarding them now as applying to the whole cycle of exercise and recovery, $W_0 = 10 \cdot 96$, $W_0 ab = 5 \cdot 32$; hence $ab = 0 \cdot 485$.

Similarly, his subject A.S.C. gave a value $ab = 5 \cdot 87/12 \cdot 4 = 0 \cdot 475$, and his subject H.L., $ab = 3 \cdot 18/6 \cdot 38 = 0 \cdot 500$. The mean of these three concordant values of ab is $0 \cdot 487$. Substituting for k and ab in the last expression for $E_{\text{max.}}$ above, we find

$$E_{\text{max.}} = 0 \cdot 24.$$

This refers to the efficiency of the whole cycle of muscular contraction, initial process and recovery: and corresponds to an efficiency in the initial process alone of about 50 per cent. (using the ratio $2 \cdot 07$ of total to initial heat found for frog's muscle). This calculation, as indeed Lupton showed, agrees well with the actual observed maximum efficiency of man. The anaerobic efficiency comes out at twice the value found above for the frog's muscle.

We are now in a position to understand why human muscles can attain so much higher a maximum efficiency than those of the frog. The expenditure of energy per second in maintaining a contraction in a frog's muscle is relatively so great (see Hartree and Hill (7), p. 147) that, for efficiency, he is of necessity reduced to contractions of very short duration. In these the rate of shortening must be rapid, or relaxation will set in before the work is done. At high speeds of shortening, however, the viscosity of the muscle reduces the work to a comparatively small fraction of its theoretical maximum. Thus the efficiency of the muscles of the frog is inevitably low. In man, on the other hand, the expenditure of energy per second in maintaining a contraction is relatively small, so that more prolonged contractions are allowable. Hence the rate of shortening can be lower, sufficiently low at any rate to reduce the effect of viscosity to comparatively small dimensions. Thus man is able to attain an efficiency about twice as high as the maximum efficiency of the frog. *The essential difference, therefore, between the two types of muscles lies in the rate at which energy must be expended to maintain a contraction.* Whether this difference be intrinsic in the muscles, or be a consequence of voluntary innervation as compared with direct electrical excitation, only future investigation can show.

The above discussion is not strictly accurate. We have neglected such factors as the Fenn effect (the change of total energy when work is performed) and the fact that the influence of viscosity in the isolated muscle, as Levin and Wyman (1) showed, does not strictly follow the simple equation (I) above. Such factors, however, are of secondary importance, and their introduction at this stage would merely complicate the issue: essentially they are of the nature of comparatively small corrections to the main result. The chief factors determining the efficiency seem clear, and the next step should be to study them quantitatively in different types of animal and muscle.

Summary.

1. The ratio $\frac{\text{work done}}{\text{total energy liberated}}$ (the "efficiency") has been examined experimentally under various conditions in the sartorius muscle of the frog. The work has been measured as the area of a tension-length curve, recorded at any desired speed by the ergometer described by Levin and Wyman.

2. In frog's muscle a high efficiency requires a contraction of short duration, owing to the great expenditure of energy involved in maintaining a contraction.

3. In contractions of short duration the work done varies with the speed of shortening, and a maximum occurs which is determined by a balance between muscular "viscosity" on the one hand and onset of relaxation on the other.

4. The maximum work done in a contraction of short duration varies with the duration in a manner similar to the total energy liberated.

5. By allowing a short interval to elapse between the commencement of the stimulus and the moment of release, a slight increase in work is secured.

6. The maximum efficiency of the frog's sartorius is independent of the temperature, and—within a narrow range of times—of the duration of the stimulus. The mean value found is 26 per cent., and the average deviation of 56 observations on 21 muscles from this mean is only 2 per cent. This refers to the initial process only: taking account of the recovery process the maximum efficiency would be only 12½ per cent.

7. This low value in the frog, as compared with man, is due to the fact that the maintenance of a contraction in frog's muscle is so extremely expensive that the highest efficiency is attained with contractions of very short duration.

Hence, to avoid the onset of relaxation, shortening must be rapid, and "viscosity" plays a large part in reducing the mechanical work performed.

8. In man, on the other hand, the maintenance of a contraction is relatively inexpensive, and the highest efficiency is attained with contractions of comparatively long duration. Hence shortening need not be rapid, and "viscosity" has a much smaller effect in reducing the mechanical work.

9. The mathematical equations defining the variation of efficiency with speed are re-stated, taking account of the fact that the duration of a contraction is somewhat greater than the duration of the corresponding stimulus.

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Leishmania Infantum *in Chinese Hamsters.*

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[PLATES 5 AND 6.]

The presence of peculiarly localised lesions in hamsters infected with *Leishmania infantum* was first observed by one of us (E. H.) in two animals kindly sent by Dr. Marshall Hertig. These had been inoculated at Peking on May 25, 1926, with a strain of *L. infantum* originally obtained in culture from Dr. Nicolle, and known as the " Infantum tunis K.A." strain. Before being sent from Peking, both hamsters were examined by liver puncture and found to be infected. On arrival at Tsinan, on July 17, the peripheral blood of both animals contained numerous parasites.

On August 25, approximately fifteen weeks after inoculation, one of these animals was killed, as it had the appearance of suffering from an intercurrent infection. The regions of the distal joints of all four limbs were enormously swollen. The testes, and also the base of the tail, were swollen and suppurating, and one of the testes contained an abscess which was discharging through the scrotum. Examination of the pus from the tail showed the presence of very large numbers of *Leishmania*. In addition, the margins of the ears and also the nose of this animal showed thickening and ulceration. All other organs of the body were normal, both in size and general appearance, and it is of interest that there was no enlargement of the spleen, which is such a characteristic feature in animals infected with typical strains of *Leishmania donovani*.

On microscopic examination, enormous numbers of *Leishmania* were found in all the ulcerated regions, and especially in the neighbourhood of the joints and in the testes. The blood and also the liver, spleen, kidney, and bone-marrow from the femur, were negative to ordinary examination, but cultures of the spleen and liver were both positive. These results seemed to indicate that the parasites were being eliminated from the visceral organs and blood, for previous examinations had shown that the liver as well as the blood were heavily infected. This view was borne out by the results of subinoculation into other hamsters.

Three giant hamsters, *Cricetulus triton*, each inoculated with a large dose of liver, spleen and kidney emulsion from this animal, remained uninfected. Four small hamsters, *C. griseus*, were similarly inoculated, and of these only one became infected, and this individual merely showed a very slight infection, which was confined to the testes. Ten other hamsters were subinoculated from the testes of this latter animal, and only one of these showed any subsequent infection, which involved the tarsal joints of both hind legs. These were heavily parasitised, and, in addition, the testes of this animal also contained a few parasites.

The second infected hamster received from Peking showed pathological appearances very similar to those of the first, the joints and tail being enormously swollen; the animal died five months after being inoculated. During life the peripheral blood frequently swarmed with parasites, and another hamster was infected by the inoculation of a few drops of blood taken from the tail. The post-mortem examination of the liver, spleen, kidney and femoral bone-marrow was negative to microscopical examination, and, in addition, the parasites had disappeared from the peripheral blood.

The swollen joints, however, and the testes contained very numerous parasites, and when these were subinoculated into two other hamsters, both acquired heavy infections, involving the joints in the manner described above. In one of these animals, a female, the peripheral blood was constantly negative; when it was killed nine months after inoculation parasites were found only in the enlarged joints and in the inguinal lymphatic glands. The liver, spleen, kidney and bone-marrow from the femur were all negative. The other animal, a male, after an incubation period of about three months, showed parasites in the peripheral blood, which rapidly increased in number. When killed, four months after inoculation, this animal, in contrast to those already described, showed a few parasites in the smears of both the liver and spleen, but as the blood was positive they may have been derived from the circulation. The joints, testes and lymphatic glands, on the other hand, all contained large numbers of parasites.

Many further passages were made with this strain in both *Cricetulus griseus* and *C. triton*, and the results of the experiments suggest that the general course of infection with this strain of *Leishmania infantum* is as follows:—

When animals are inoculated intraperitoneally, the parasites may be found within twelve days in the lymphatic glands. Subsequently, they invade the liver, spleen and other organs, giving rise to a generalised infection, while frequently they appear in the blood stream. Later, the organisms gradually disappear from the viscera and become localised in the tissues surrounding one or more of the distal joints of the legs, and in the tail. The testes are also parasitised and occasionally show abscess formation; the ears and nose of a certain number of animals also show thickening followed by ulceration. The parasites are present in all these lesions, usually in very large numbers, and either absent or very difficult to find in other situations. In only one individual out of 43 inoculated was any enlargement of the spleen observed, and this animal showed a heavy infection of both liver and spleen six months after inoculation. When animals were inoculated intra-testicularly, the course of the resulting infection was similar to the above.

Three dogs inoculated with this strain, one with culture forms and the other two with emulsions of the organs of infected hamsters, showed no signs of infection after prolonged periods of observation, and their organs were negative on *post-mortem* examination.

The course of the infection in hamsters inoculated with the Chinese strain of *Leishmania* differs markedly from that just described. The liver, spleen and bone-marrow all become infected, and as the disease progresses these organs show increasing numbers of parasites. No obvious peripheral lesions such as tumefaction of the joints, or abscess formation, were ever observed in animals infected with this strain, and the extent to which other organs were involved seemed to depend on the duration of the disease. The differences in the course of the disease, and the lesions produced, indicate that the Chinese and Tunisian strains of *Leishmania* may be sufficiently distinct to be regarded as different varieties, if not species.

Histopathology.

The occurrence of these lesions in hamsters infected with this same strain of *Leishmania* was briefly recorded by Young and Hertig (1927), and as the peculiar localisation of the parasites in hamsters seems to be a characteristic feature of this infection, it seemed of interest to study the pathological changes taking place in the testes, and especially in the joints. The material was fixed in Bouin's picro-formol and stained with hæmatoxylin, or hæmalum, and eosin, or with Giemsa.

The Testis.—Testicular enlargement in animals infected with *Leishmania* was described by Laveran (1917) in the case of mice inoculated with *L. tropica*, which also showed the same periarthritic lesions. Hamsters infected with a strain of *L. tropica* obtained by Dr. C. W. Young in Irak never showed any of these lesions, although in rare instances the organisms were recovered from the testes. On the other hand, when these animals were inoculated with the Tunis strain of *Leishmania infantum*, the testes always showed a distinct enlargement, and in later stages abscess formation was sometimes observed followed by discharge through the scrotum.

Microscopic Examination.—Meleney (1925) briefly records the presence of parasites in small numbers in the connective tissues of the testis, epididymis, and seminal vesicles of hamsters infected with the Chinese strain of *L. donovani*. In addition, human cases were examined. In both hosts the parasites were found only in clasmatocytes situated in the loose, interstitial tissues. Shortt (1923) also described parasites in the interstitial " Cells of Leydig " of monkeys infected with the Indian strain of *Leishmania.*

Sections were taken both transversely and through the long axis of the testis and epididymis of a typical case of enlargement of these organs in a hamster infected with *L. infantum*. Examination under the low power showed a very great proliferation of the cells in the inter-tubular tissues (fig. 1, Plate 5), which is in marked contrast to that commonly seen in hamsters infected with *L. donovani* (fig. 4, Plate 5). It should be noted that the duration of the infection does not account for this difference, as in the two cases illustrated the former had been inoculated about twelve months previously, whilst the latter had been infected for fifteen months.

The interstitial cells were composed of large mononuclears (polyhedral cells), the cytoplasm of which was intensely eosinophile, numerous small connective-tissue cells, and a few plasma cells. No parasitised clasmatocytes were seen in this region, although there can be little doubt of their presence at earlier stages of the disease. There was no evidence of any decrease in spermatogenesis, for in certain areas were large masses of fully developed spermatozoa, completely filling the seminiferous tubules, and also active developmental stages.

In certain areas the lumina of the tubules were obliterated and filled with numerous polymorphonuclear cells, obviously the result of some secondary inflammatory condition. The sperm mother-cells of the outer zone of these inflamed tubules sometimes contained numerous *Leishmania* bodies (figs. 2 and 3, Plate 5). Many tubules showed no inflammatory condition, but often the cytoplasm of the sperm mother-cells contained clumps of parasites and some of these infected cells occurred free in the lumen. As far as we are aware, this is the first record of these parasites occurring in the sperm mother-cells of the testis.

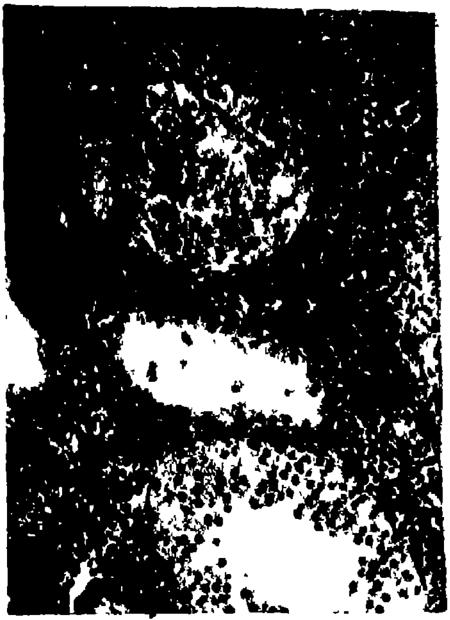
No marked changes were observed in the interstitial cells of the epididymis, and no parasites were found either in the intertubular spaces, or in the lining cells of the tubules.

Periarthritic Lesions.

Most animals ultimately showed enlargement of the regions surrounding the carpal and tarsal joints. Sections through the foot, including the tarsals, metatarsals and the distal ends of the tibia and fibula, showed that the swelling of the joint was mainly caused by a very great proliferation of cells immediately around the ankle joint. These cells were mainly parasitised clasmatocytes which had invaded the *cutis vera* and extended down to the periosteum of the bones and the ligaments in the neighbourhood of the joint (fig. 5, M, Plate 6). On the anterior aspect of the articulation of the tibia and fibula with the tarsals, there was a marked proliferation of macrophage tissue in the *cutis vera*, which, by pressure, had raised the epidermis. This multiplication of cells tapered off on each side of the joint. Posteriorly, there were masses of heavily parasitised cells, also originating in the *cutis vera*, and in this region extensive hæmorrhages were observed (see fig. 7, Plate 6). The cavity of the joint did not seem to be invaded with parasites, but enlarged fringes of thickened synovial membrane projected into the cavity. The tissue between the muscles and ligaments of the tarsals and metatarsals contained masses of parasitised clasmatocytes. We have never observed any parasites in the tissues surrounding the bones of the toes. The bone-marrow of the lower ends of the tibia and fibula was almost entirely replaced by parasitised clasmatocytes (fig. 8, C, Plate 6).

It is curious that these parasitised cells do not seem to extend proximally for any distance up the bone-marrow, and except at the early stages of the disease, during which the infection is more generalised, we have very rarely observed any parasites in the bone-marrow of the femur. On the other hand, the bone-marrow of the tarsals and metatarsals contained large numbers of parasites, but none were observed in the phalanges.

The main interest of the observations with this strain lies in the fact that, after first producing a general visceral infection, the parasites become localised in these regions of the body. The subcutaneous tissues of the body of infected hamsters, including the abdomen and back, were examined, but parasites were never observed except in the periarthritic regions, or unless obvious lesions were present, such as on the margins of the ears. This is in marked contrast with hamsters infected with the Chinese strain of *Leishmania donovani*, in which a widespread occurrence of parasites in the subcutaneous tissues of the whole body is a common feature in late stages of the infection.



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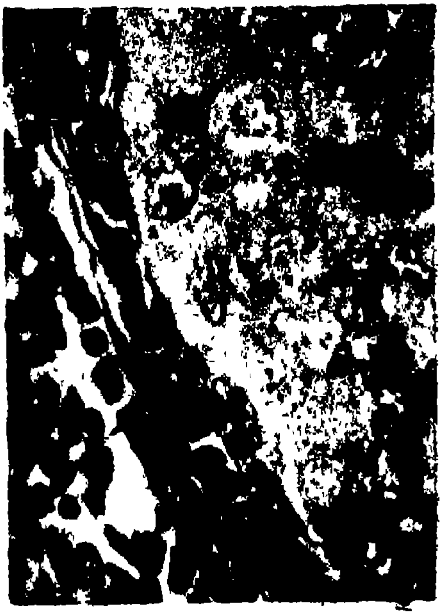

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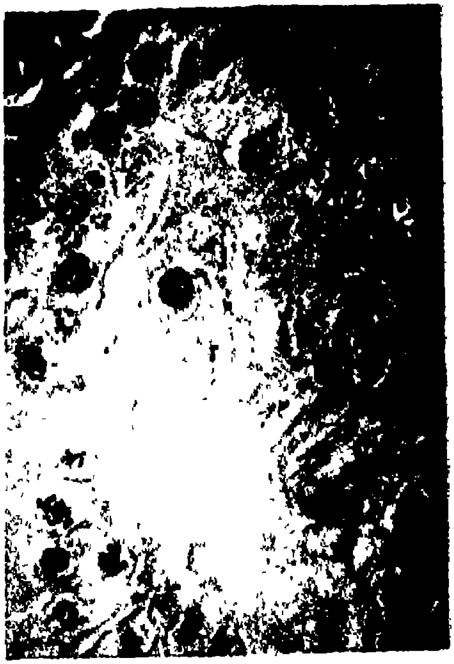

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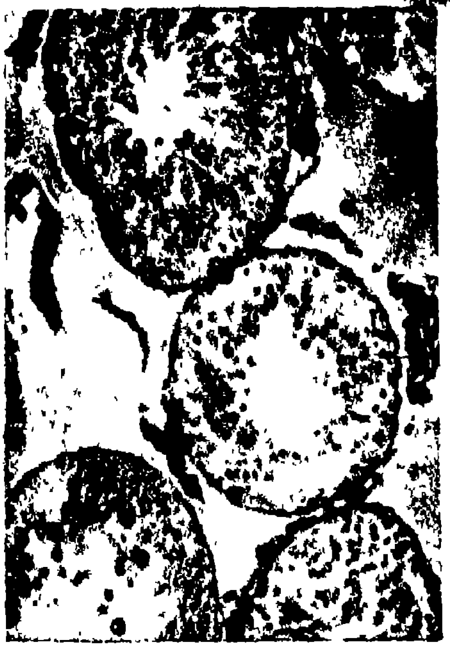

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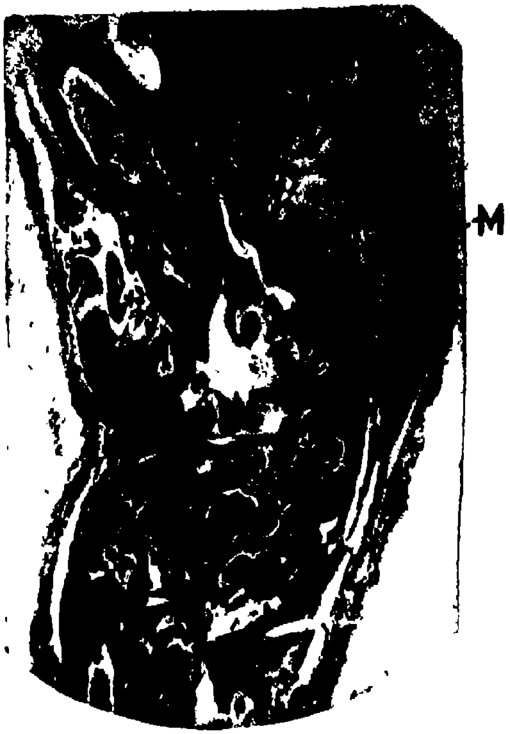

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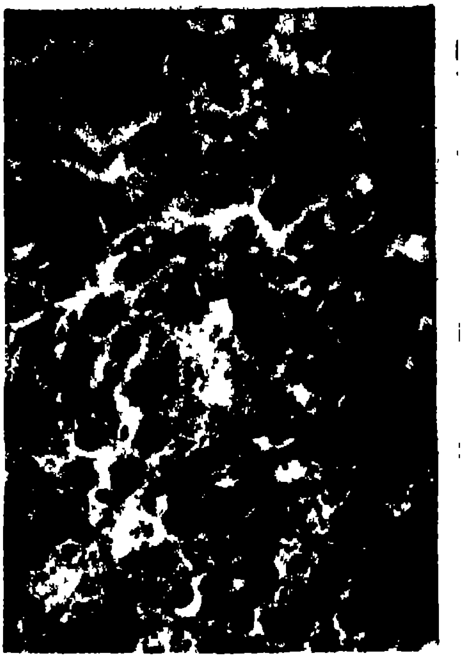

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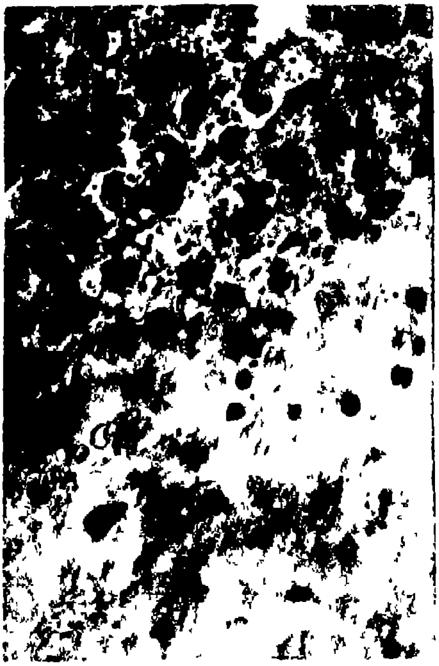

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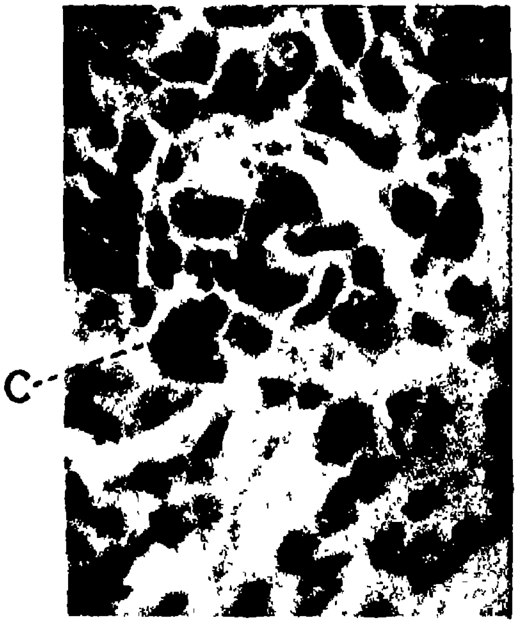

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DESCRIPTION OF PLATES

Plate 5

Fig 1 (× 150) Longitudinal section through testis of hamster infected with *Leishmania infantum* The tubule on the top of the photograph shows obliteration of the lumen by a mass of polymorphonuclear cells Note the very great proliferation of cells in the intertubular regions

Fig 2 (× 800) More highly magnified photograph of the left margin of upper tubule outlined in fig 1, showing masses of *Leishmania* in the cytoplasm of the sperm mother cells

Fig 3 (× 450) Section through a seminal tubule of the same hamster, with no secondary infection of the lumen, but showing invasion of the sperm mother cells by *Leishmania*

Fig 4 (× 800) Section of the testis of a hamster infected with *Leishmania donovani* for 15 months, for comparison with fig 1 In this case there is no increase in the number of intertubular connective tissue cells, although occasionally parasitised cells were observed

Plate 6

Fig 5 (× 20) Longitudinal section of the ankle of a hamster infected with *L infantum*, on the right showing a subcutaneous mass of infected tissue M, extending down to the ligaments and periosteum

Fig 6 (× 800) More highly magnified photograph of area outlined in fig 5 showing the parasites in the clasmatocytes forming the mass of infected subcutaneous tissue

Fig 7 (× 800) Another portion of the section showing a hæmorrhage in the subcutaneous tissue, surrounded by parasitised cells

Fig 8 (× 800) Section through the lower end of the fibula of the same hamster, showing the replacement of the bone marrow by parasitised clasmatocytes C

The Development and Morphology of the Gonads of the Mouse. Part III.—*The Growth of the Follicles.*

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(Communicated by Prof. J. P. Hill, F.R.S.—Received May 19, 1928.)

(From the Department of Anatomy and Embryology, University College, London.)

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I. INTRODUCTION.

The present paper consists of a series of records of the growth of the oocyte and follicle in the ovary of the adult mouse. Wherever it has been possible, these records have been put in mathematical form. The results show clearly, amongst other things, that the major part of the growth of the Graafian follicle takes place after the contained oocyte has attained its maximum size. This fact is of considerable interest in connection with the evolution of the mammalian follicle and its physiological rôle.

The author would like to take this opportunity of expressing his thanks to "Student" for advice and criticism of the statistical treatment of the results, and to Prof. J. P. Hill, F.R.S., and Dr. A. S. Parkes for the interest they have taken in the work.

The histological expenses were defrayed by a grant from the Government Grants Committee of the Royal Society, for which the author's thanks are due.

II. Description.

1. *The Growth of the Oocyte.*

The smallest oocytes found in the adult mouse ovary are situated just beneath the germinal epithelium and are surrounded by a few flattened follicle cells. The oocytes only measure 13 μ in diameter, approximately. They are approximately the same size as the average of the primordial germ-cells found in and near the germinal ridge of the 10-days *post-coitum* embryo, which measure from 10·5 to 16·5 μ in diameter. The smallest oocytes in the adult ovary, enclosed in primordial follicles, are always situated just beneath the germinal epithelium, with at most three or four cells between. Their nuclei however, never exhibit any contraction figures and are always in the dictyate condition.

Oocytes at all stages of growth between these small oocytes and the full-grown oocytes, measuring approximately 70 μ in diameter, can be found in one ovary. It is impossible to ascribe a definite time limit to this growth stage, but it is probably fairly gradual. Once the oocyte has attained its maximum size it appears to remain, without growing more, until ovulation is almost due.

The nucleus increases in size with the growth of the oocyte. The diameters of a large number of oocytes and their nuclei were measured and the results treated statistically. The measurements were made from camera-lucida drawings at known magnifications. The diameter of the outline of each oocyte and its nucleus was measured in two directions, at right angles to each other, and the average of each pair of measurements was taken to be the true diameter. The results are shown in the form of a correlation table (Table I).

Table I.

Diameter of nucleus. / Diameter of oocyte.	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80 μ
30·0–32·5 μ													1	
27·5–30·0											1	2	13	1
25·0–27·5											3	16	18	2
22·5–25·0									3	9	6	10		
20·0–22·5								6	2	1	2			
17·5–20·0						3	3	5	2	2				
15·0–17·5					1	4	2	1						
12·5–15·0		1	4	5	5									
10·0–12·5	3	4	6											
7·5–10·0	7													

The distribution of these data is not normal for two reasons: (1) The number of oocytes of a given size present in any ovary decreases as the size increases owing to degeneration taking place at all stages; and (2) oocytes of suitable sizes were selected for convenience. From these considerations it is obvious that the coefficient of correlation will have no meaning apart from the data from which it has been derived. Since, however, the data were only selected in one direction—*e.g.*, size of oocyte—the regression formula will supply a true value for the relation between the size of the nucleus and the size of the oocyte. The regression function, calculated from these data, is $Y = 5\cdot577 + 0\cdot297\,x$, where x is the given diameter of the oocyte and Y is the mean value of the diameters of the nuclei for each value of x. Entering the table of t (Fisher (5)) with $t = 60\cdot69$, and $n = 152$, P is obviously much beyond 0·01 and therefore the regression is decidedly significant; the growth of the nucleus and that of the oocyte undoubtedly proceeding simultaneously. This regression function is linear and is represented graphically in fig. 1, where the mean value of each array is also shown as an ×.

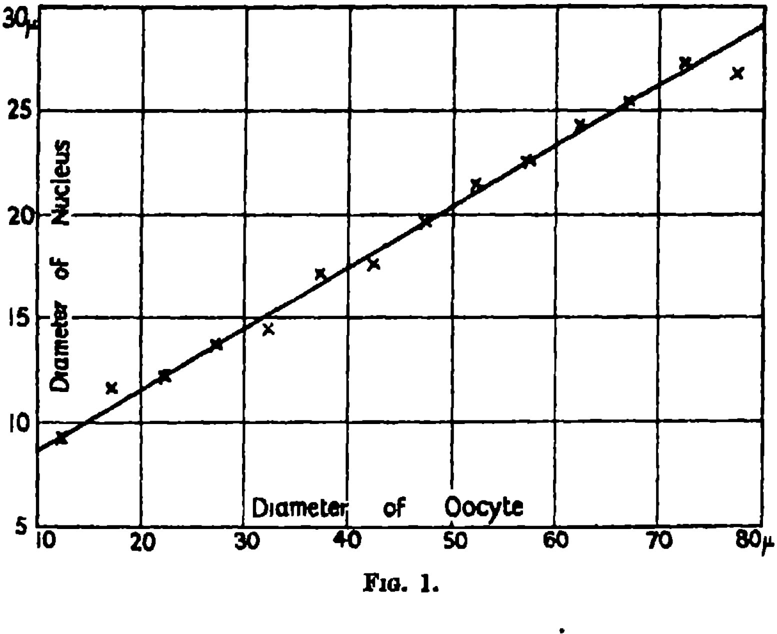

Fig. 1.

2. *The Growth of the Oocyte in Relation to the Growth of the Follicle.*

The diameters of a large number of follicles and their oocytes were measured. The method was the same as in the case of the oocytes and their nuclei. The results are shown in the form of a correlation table (Table II). These were treated as a regression, for the same reasons as in the previous section, of size of oocyte on size of follicle. The distribution seemed to justify dividing the

Table II.

Diameter of oocyte. \ Diameter of follicle	0–25	25–50	50–75	75–100	100–125	125–150	150–175	175–200	200–225	225–250	250–275	275–300	300–325	325–350	350–375	375–400	400–425	425–450	450–475	475–500	500–525	525–550	550–575	575–600	600–625	625–650 μ
80–85 μ													2			1	1									
75–80								1		3	1	4	3	1	2	1	2	1	1	2		1		1		
70–75						2	3	1	5	5	15	13	7	10	6	8	8	10	5	2	5	1	2	1	1	
65–70						1	4	3	10	11	15	6	10	2	5	9	9	5	5	2	1	3		1	1	1
60–65					9	2			1		2	1	1	3	1				1	1		1	1			
55–60				8	3								1													
50–55				6																						
45–50			1	6																						
40–45			2																							
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correlation table into two parts, one covering the range of follicle diameters from 0 to 150 μ and the other from 150 to 650 μ. The regression function for the first part, which is linear, is $Y = 5\cdot795 + 0\cdot502\,x$, where x is the given diameter of the follicle and Y is the mean diameter of the oocytes for each value of x. Entering the table of t (Fisher (5)) with $t = 37\cdot4$, and $n = 55$, P is obviously much beyond 0·01 and therefore the regression is decidedly significant. The regression function for the second part, which is also linear, is $Y = 69\cdot54 - 0\cdot0025\,x$. Entering the table of t (Fisher (5)) with $t = 1\cdot077$, and $n = 251$, P is approximately 0·3 and therefore the regression is not significant (*i.e.*, there is no significant alteration in size of the oocyte during the growth period of the follicle covered by the regression line). These regression functions are represented graphically in fig. 2, where the mean value of

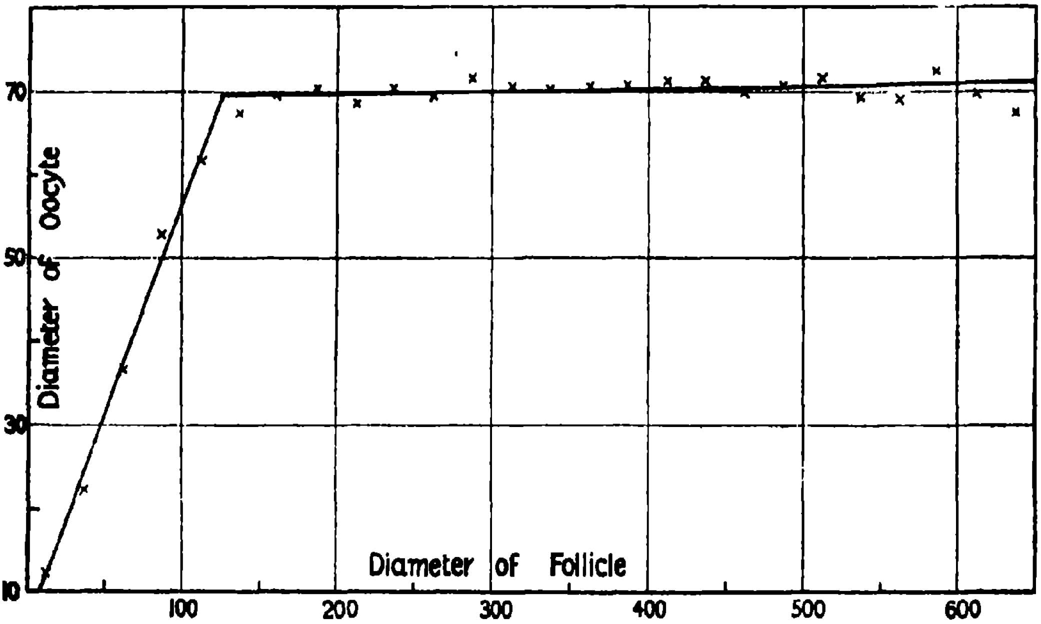

Fig. 2.

each array is also shown as an ×. The two regression lines intersect at approximately 125 μ (diameter of follicle). These results show that the increase in the size of the oocyte bears a direct and significant relation to the size of follicle until it attains a size of approximately 70 μ in diameter, when the follicle is 125 μ in diameter. Subsequently, there is no significant growth of the oocyte, and the growth of the follicle may be said to be independent of it and can be represented by a horizontal line in the graph.

3. *The Maturation of the Oocytes.*

The full-grown oocyte of the mouse with the nucleus in the resting dictyate stage, fixed in Bouin's fluid, measures 70·96 μ ± 0·25 μ in diameter. A reduction in size appears to occur during maturation. Ten oocytes, exhibiting the first polar spindle, from the ovaries of one mouse measured 66·9 μ in diameter, the largest being 71 μ in diameter and the smallest 63·5 μ. Three ova, from the Fallopian tubes of two mice, which in each case contained ♂ and ♀ pronuclei unfused, and in which the second polar body had been extruded, measured 55·5, 56·0 and 57·3 μ in diameter respectively. These three ova had, however, been fixed in Carnoy's fluid, and the difference in fixation might account in part for the difference in size. In any case the numbers observed are too small to supply a significant statistical difference, although they suggest that a reduction takes place during the first maturation division and a further reduction during the second maturation division. The cytological changes in the ovum of the mouse during maturation and fertilisation have been described by Sobotta (11).

4. *The Growth of the Follicle.*

The smallest oocytes, situated just beneath the germinal epithelium in the adult ovary, are only surrounded by a few flattened epithelial cells—the primordium of the membrana granulosa. These primordial follicles only measure about 17·5 μ in diameter. Slightly larger oocytes, about 14 μ in diameter, are surrounded by a single layer of more or less cubical epithelial cells, and are situated in or immediately beneath the tunica albuginea. These follicles measure about 28 μ in diameter. The development of the various structures of the follicle is shown in tabular form in Table III (p. 264).

The follicles with a single layer of epithelium reach their maximum size at about 36 to 40 μ in diameter, with the contained oocytes 22 to 24 μ in diameter. By the time the oocyte has attained a size of about 30 μ and the entire follicle about 50 μ, the epithelium shows distinct signs of becoming double-layered. The double-layered condition is fully established in follicles of 95 to 100 μ in diameter with oocytes 55 to 60 μ. The follicular epithelium may now be considered to form a membrana granulosa. The growth of the follicle proceeds rapidly and changes from two to three cells thick by the time the oocyte has completed its growth and has attained a size of, on an average, 70 μ in diameter. These follicles measure about 125 μ in diameter.

Table III.

Diameter of follicle in μ.	Condition of oocyte.	Condition of follicle.
17·5	13 μ in diameter	Single layer of flattened epithelial cells.
28	20 μ ,,	Single layer of cubical epithelial cells.
50	31 μ ,,	Follicular epithelium becoming two cells thick.
100	58 μ ,,	Membrana granulosa two cells thick. Theca interna beginning to differentiate.
125	Oocyte full grown. 70 μ in diameter	Membrana granulosa becoming three cells thick and mitoses in it most frequent.
200	70 μ in diameter	Definite theca interna formed. Antrum first appears.
380	70 μ ,,	Beginning of oestrous cycle ending in ovulation.
410	70 μ ,,	Œstrus stimulation effected. Rapid growth-phase starts.
530	70 μ ,,	Secondary liquor folliculi appears. Mitosis in membrana granulosa stops.
550	1st polar spindle formed	Oocyte in corona radiata floating free in the antrum. About to ovulate.

Up to this time the growth of the follicle and of its oocyte have been correlated. The subsequent growth of the follicle, however, is entirely independent of the oocyte, which has reached its maximum size, and is due in part to the growth of the membrana granulosa and the development of the theca interna, but chiefly to the formation and increase in size of the antrum.

The epithelial cells of the membrana granulosa grow rapidly by mitoses at all stages of follicular development. They exhibit mitoses even in the small follicles, where they constitute a single-layered epithelium. In follicles in which the membrana granulosa is passing from the double- to the triple-layered condition many mitoses are present. They occur frequently in all stages until maturation is approaching. Mitoses then become less frequent, except in the region of the discus proligerus, where mitotic figures are abundant, and, finally, in follicles containing mature oocytes in process of polar-body formation, practically none can be observed.

The bodies of Call and Exner, so common in the membrana granulosa of the follicles of man, cat, rabbit, etc., do not occur at all in the mouse, nor is any comparable stellate arrangement of the cells ever observable.

The theca interna does not exist as a separate layer, in small follicles, which are surrounded by a coat of undifferentiated connective-tissue fibroblasts. Elements that would ultimately form the theca interna can first be distinguished in this primitive theca of follicles approaching 100 μ in diameter. The cells are larger and more glandular in appearance than the surrounding fibroblasts, among which they are scattered. The theca interna cells and a few fibroblasts form a definite, if somewhat irregular, layer within the more fibrous theca externa, in follicles 200 μ in diameter.

At this stage the theca interna measures about 20 to 25 μ in thickness. It is rather irregular, but is, on the whole, as well developed on the side next the periphery of the ovary as on that away from it. At all stages it has numerous undifferentiated connective-tissue elements, as well as blood capillaries and lymph channels, scattered between the large, apparently glandular cells. It increases in thickness as follicular development progresses, except on the peripheral surface of the follicle. In the latter region it becomes thinned out as maturation is completed. In the follicle ready to ovulate it attains a maximum thickness of 30 to 40 μ on the side away from the periphery, but is very thin on the peripheral surface, where the membrana granulosa is only covered by a thin layer of fibroblasts, one or two cells thick, derived from the theca and tunica albuginea, and by the germinal epithelium. This peripheral thinning of the coat of the follicle allows of rupture being effected more easily. The large flattened glandular cells of the theca interna exhibit many mitoses in the smaller follicles, but very few are present in the later stages of maturation.

The membrana propria is not well developed in the mouse, and consists of a light, fibrous network, between the membrana granulosa and the theca interna.

The theca externa exhibits mitoses at all stages of follicular growth. It is thinner and more compact in the larger follicles, probably owing to stretching and compression exerted from within by the growth of the follicle.

The antrum first appears as an irregular fluid-filled cleft in the middle of the membrana granulosa on one side of the oocyte, in follicles about 200 μ in diameter. This cleft enlarges as the follicle grows and comes to form a half-moon shaped cavity in the membrana granulosa with its concavity directed towards the oocyte. As maturation proceeds the antrum increases in proportion and becomes more or less the same shape as the follicle, with the discus proligerus and its contained oocyte projecting into it. The membrana granulosa enclosing the antrum, except on the side of the discus proligerus, is more or less uniform in thickness, varying from about 55 to 65 μ, at all stages of growth. The diameter of the antrum at right angles to the axis passing through the ooyctes and the centre of the follicle can therefore be said to be roughly the diameter of the follicle less 120 μ. This approximation obviously is more accurate for larger follicles, where the discus proligerus on account of its relatively smaller size does not seriously complicate the estimate.

5. *The Growth of the Follicle in Relation to Œstrus.*

It is well known that the growth of the follicle is extremely rapid at the end, and that the main increase in its size takes place during a short period ending in ovulation. It has been shown elsewhere, in collaboration with Dr. Parkes (3), that the follicles, which will ovulate at the following œstrous period, in the ovary of an unmated mouse are on an average only 380 μ in diameter at the beginning of the œstrous cycle. The follicles reach a maximum size of, on an average, 550 μ in diameter immediately before rupturing. Further, the follicles undergo comparatively little growth until 48 hours before the onset of the œstrous cornification, marking the period at which ovulation will take place The growth, therefore, is increasingly rapid as œstrus approaches, and the follicles increase 45 per cent. in diameter in the last 48 hours.

A definite group of large follicles which will ovulate at the ensuing œstrous period can generally be distinguished in the ovaries of an unmated mouse. This group is, however, not very clearly defined, and sometimes the maturing follicles cannot be distinguished with certainty from those destined to ovulate at subsequent œstrous periods, if the ovaries were taken from an animal killed at the beginning of a cycle.

It was thought that several such groups of follicles, destined to mature at subsequent œstrous periods, might be present in the ovaries at one time. In consequence all the follicles measuring 75 μ in diameter and over in one ovary were measured and counted. The size distribution of these was such as to afford no prospect of demonstrating by statistical methods a series of follicle-size groups in the ovaries of unmated mice. It may be assumed, as the contrary cannot be demonstrated, that the follicles in the ovary of the unmated mouse are, as regards size, distributed at random, excepting those destined to rupture at the ensuing œstrus.

6. *The Maturation of the Follicle in Relation to Œstrus.*

The follicles undergo various changes immediately before rupture. The ovaries removed from a mouse 6 to 26 hours before it came into œstrus (OVS 41) contained 12 large follicles, averaging 530 μ in diameter, the largest being 576 μ and the smallest 464 μ in diameter. These follicles differed little, except in size, from smaller ones. The oocytes had dictyate nuclei. The discus proligerus was still attached to the wall of the follicle, but in its neighbourhood the coagulated liquor folliculi, situated in the antrum and between the membrana granulosa cells, appeared more glairy and granular than elsewhere in the follicles, or than in smaller follicles. This thick liquor is that termed the secondary liquor folliculi. The ovaries removed from a mouse killed during late pro-œstrus or early œstrus (CLC 114) contained 11 large follicles, averaging 550 μ in diameter, the largest being 626 μ and the smallest 475 μ in diameter. The oocytes in all these follicles exhibited the first polar spindle, with the chromosomes in the equatorial plane or beginning to draw apart to either pole of the spindle. In these follicles the discus proligerus, containing the oocyte, had become separated from the wall of the follicle and floated free in the liquor-filled antrum. The cells of the discus proligerus had become altered in character. They were arranged in a stellate manner around the oocyte and were drawn out in a radial direction forming a corona radiata. The secondary liquor folliculi was more abundant than in the previous example and was present between the cells of the discus. Coupled with these changes the thinning of the follicle wall at the periphery, described above, indicated that the follicles were about to rupture.

The material from two mice exhibited ova in the Fallopian tubes. These ova had extruded the second polar body in each case and contained both male and female pronuclei approaching to each other. Ovulation in these had obviously occurred some hours previously, as the ova had reached the tubes and been fertilised. Both these animals had been killed on the morning on which the vaginal plug had been found. Mice usually copulate early in the night following the onset of œstrus. Further, œstrus usually commences in the evening. Consequently these animals cannot have been on œstrus more than 24 hours, and probably not so long.

Further, the ovaries of several mice killed during the first 12 to 18 hours after the onset of œstrus were available. All these exhibited young corpora lutea in various stages of formation from newly ruptured follicles. Unfortunately the tubes containing the ova of these were not available. They serve, however, to confirm the previous observations.

From these cases it is clear that ovulation in the mouse takes place during late pro-œstrus or very early œstrus. It probably occurs about the time when pro-œstrus changes into œstrus, and usually not more than 6 hours either side of this time. Within these limits a certain amount of variation may be expected to occur in different individuals and in different follicles in any individual. The material, however, is not sufficient to afford any further estimate of this variation. The actual size of the follicles at the time of rupture probably varies considerably also, as the case described (CLC 114) seems to indicate.

7. *The Number and Distribution of Mature Follicles in the Ovaries at Œstrus.*

The average number of follicles which mature in the two ovaries at each œstrous period can be estimated indirectly from the average size of litter born and the average pre-natal mortality. The litter size has been shown by Parkes (9) to be 6·34. It is obvious from these figures that at least seven oocytes are matured at each œstrus.

The required figure can also be arrived at by counting the number of corpora lutea formed at each period, or, directly, by counting the number of maturing follicles in serial sections of the ovaries. The latter method is somewhat uncertain immediately after an œstrous period, as the maturing follicles are not sufficiently clearly defined by their sizes from the largest of the other follicles. The following table, however, contains the results from nine clear cases:—

Table IV

Reference number.	Number of maturing follicles	Distribution.
CLC 114	11	7/4
OVS 125	11	—
OVS 41	12	6/6
OVS 39	9	5/4
OVS 49	8	—
OVS 96	8	4/4
OVS 52	9	6/3
OVS 40	9	7/2
OVS 30	7	4/3
9	84	

These give an average of 9·3, which corresponds to the estimate arrived at from the size of litters. The problem of whether the maturing follicles are distributed at random between the two ovaries or tend to be equally distributed between them is of interest, but difficult to demonstrate on account of the amount of material required. In three of the seven cases recorded, the distribution was unequal. The material is insufficient to show a significant difference from a random distribution.

8. *The Degeneration of Follicles.*

Many follicles of all sizes can be found degenerating in the ovaries of the mouse at any time after puberty and at all stages of the œstrous cycle. It is, however, very difficult to estimate this owing to being unable to tell for how long a degenerating follicle is distinguishable as such. In the case of the primordial follicles it is known (4) that they can completely disappear after treatment with X-rays in 24 to 48 hours. The degeneration of larger follicles is, however, much slower. In one typical ovary examined 128 normal follicles over 100 μ in diameter were counted and at least 66 follicles in process of degeneration which had been probably all over 100 μ in diameter before degeneration set in. It is, therefore, probable that a very large percentage of the follicles are eliminated at all stages up till the time of ovulation. This percentage appears to vary considerably in different individuals and to be much greater in poor conditioned and unhealthy than in sound animals.

III. Discussion.

The Oocytes.

The ovary of the adult mouse has never been observed to contain any oocytes exhibiting the prophase spireme and synaptic figures ((2) for previous literature). There can therefore be no doubt that oocytes do not pass through these stages in the adult mouse, as do those described in the Lemur by Gerard (6). This is held by many as definite proof that no neoformation of oocytes occurs in the adult mouse. Such a neoformation of oocytes from the cells of the germinal epithelium has been described by several authors, notably Allen (2), who admit that it takes place without going through the typical nuclear phases of synapsis. In the present paper no attempt has been made to solve this problem, owing to the inherent difficulties of estimating the number of small oocytes in each of a large series of ovaries with sufficient accuracy to obtain statistically sound results. In this connection it is significant, however, that in the ovarian tissue regenerated in the mouse after complete double ovariotomy (10) oocytes were present but never exhibited the prophase figures.

Throughout the entire growth of the oocyte the nucleus is in the dictyate stage, and bears a constant relation to the size of the cell. The oocyte, after it has attained its maximum size, appears to be able to remain for an indefinite time before it enters on the maturation stages. The material described supports the view that a slight decrease in the size of the oocyte takes place at the time of the formation of the first and second polar bodies.

Ovulation in the mouse appears to take place during late pro-œstrus or early œstrus. This is earlier than Allen (1) considered to be the case, as he stated that it did not take place before the end of œstrus in the mouse, at the same time as Long and Evans (8) described it as occurring in the rat. The material described in this paper, however, admits of no other conclusion.

The number of follicles maturing in the nine pairs of ovaries dealt with averaged 9·3. Parkes (9) has shown that the average size of litter for the colony from which these are drawn is 6·34. It is therefore apparent that roughly two-thirds of the follicles which mature produce ova which survive till birth. This figure is slightly larger than that arrived at by Long and Evans (8) in the case of the rat, but is quite comparable. They found that the average number of corpora lutea produced in an animal at each œstrus was 10·8, while the average number of ova in the oviduct was 9·6. The average size of litter in their colony was 6·4.

The Follicles.

The results obtained on the relative growth of the follicle and the contained oocyte show that a relation exists up till the time that the oocyte has attained its maximum size. This, however, takes place while the follicle is still comparatively small (125 μ in diameter approximately), and the subsequent growth of the follicle is not correlated with growth of the oocyte, which remains the same size. This conclusion, which has been quoted elsewhere (3), is of extreme importance in considering the morphological and physiological significance of the mammalian Graafian follicle. At the time when the oocyte has completed its growth, and therefore requires less nutrition, the follicle has a membrana granulosa composed of only three layers of cells, no traces of an antrum containing liquor folliculi, and scarcely any differentiated theca interna cells.

All the characteristics of the mammalian Graafian follicle are thus developed after the follicle has supplied the oocyte with the nutrition necessary for its growth. It may therefore be concluded that the mammalian Graafian follicle has been evolved for some purpose other than the nutrition of the oocyte. Three possible explanations occur: the Graafian follicle as such has been evolved [1] as an endocrine organ, [2] to prepare for the rapid formation of the corpus luteum, or [3] as a mechanical adaptation to effect ovulation and the transference of the ova to the tube. Regarding the first of these possibilities the work of Parkes and the author (3) has shown that the Graafian follicle is not essential for the production of the hormone œstrin, which causes œstrus, and is probably not concerned at all in its production. This fact leaves us without any definite endocrine function to ascribe to the follicle.

The Monotreme follicle as shown by Hill and Gatenby (7) has no antrum, but the egg is large and yolk-laden. The fluid-filled antrum is thus associated only with the microlecithal eggs of the higher mammals. It may be, therefore, that it has been developed as a mechanical means of effecting follicular rupture by internal pressure in microlecithal forms, where the ovum itself would find difficulty in bursting through the follicle on account of its small size. It is also possible, as has been suggested elsewhere (3), that the liquor folliculi is of assistance in washing the ovum into the tube. At the time of ovulation the ovarian capsule and the tube in the mouse are distended with fluid. The amount of liquor folliculi liberated would not be sufficient to account entirely for this distention, but would probably materially assist in producing it.

Finally, the production of the large Graafian follicle may be a preparation for the formation of the corpus luteum. However, in the Monotremes, in which no antrum is formed, a corpus luteum is produced. In the latter case the ruptured follicle with its cavity is sufficiently large to develop speedily a large corpus luteum. This would scarcely be so in the higher mammals, unless the follicle grew beyond the size required to maintain the egg and attained the dimensions it does by the development of the large antrum.

At present it seems impossible to decide which of these theories is the true one. It may be that several functions are performed by the distention of the Graafian follicle with liquor folliculi and by the cells of the theca interna. Whatever these functions may be, the nutrition of the oocyte would not seem to be one of them.

IV. SUMMARY.

1. During the growth stage, the diameter of the nucleus bears a direct relation to the diameter of the oocyte.

2. The diameter of the oocyte bears a direct relation to the diameter of the follicle during the growth of the former. The oocyte attains its maximum size of 70 μ in diameter when the follicle is 125 μ in diameter.

3. The main growth of the follicle, and the formation of the theca interna and the antrum, take place after the oocyte has completed its growth.

4. During the maturation divisions the oocyte is reduced in size. Fertilised ova only average 56 μ in diameter.

5. The follicle grows rapidly, chiefly by enlargement of the liquor-filled antrum, during the two days immediately prior to the œstrous period and presumably in response to the production of œstrin.

6. Ovulation takes place during late pro-œstrus or early œstrus. The average number of follicles maturing at each œstrous period in one animal is 9·3.

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The Effect of Temperature on the Permeability of Protoplasmic Membrane.

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The effect of temperature on permeability of plant cells has been studied by numerous investigators. Stiles (8) gives a comprehensive summary of the existing work on the subject. The data regarding the effect of temperature on permeability to solutes are neither as numerous nor as conclusive as those for the intake and excretion of water. Pfeffer (1886) and Collander (1921) have observed the intake of dyes (8). Rysselberg (1901) and Lepeschkin (1905) have investigated the permeability of epidermal cells to glycerol carbamide and potassium nitrate at different temperatures. To correlate the change of permeability with positive and negative thermotropism Eckerson (1914) observed the molecular concentration of sucrose, glucose and potassium nitrate solutions, needed to induce slight plasmolysis in root cells at different temperatures (1). Stiles and Jorgensen (9) have studied the effect of temperature on absorption of hydrogen ion by storage tissues.

In all the above investigations only the intake of solutes by cells, and that under environmental conditions unnatural to cells, has been studied. So far as I am aware, Blackman and Paine (3) are the only investigators who have studied the exosmosis of the normal cell electrolytes by measuring the change of conductivity of the water in which a pulvinus of *Mimosa pudica* was placed.

In the following investigation an attempt has been made to study the effect of temperature on the permeability to electrolytes of a tissue by measuring its electric resistance at different temperatures, while kept otherwise under normal conditions. It is assumed (2) that the high electric resistance of a plant tissue is due to the semi-permeability of the cell-membrane and that the resistance decreases with increased permeability of the membrane, and *vice versa.*

The variation of electric resistance of a plant tissue may be brought about by—

(*a*) change of permeability of the plasma membrane to the enclosed ions,
(*b*) change in the degree of dissociation of the electrolytes in the cell sap and the mobility of the ions, and
(*c*) chemical changes leading to a variation in the concentration or alteration in the nature of the free ions.

Apart from its direct effect on the velocity of migration of ions, variation of temperature may have an effect on (*b*) and (*c*). Therefore, to correlate the observed variation of electric resistance of a tissue with permeability, these factors should be considered. The effect under (*c*) is negligible, since the concentration of electrolytes within the plasma membrane is very high, as seen by the low resistance when the tissue is killed by chloroform or heat. From the resistance of a killed tissue at different temperatures, the influence of temperature on the degree of dissociation of the electrolytes and the mobility of the ions can be determined, since the electrolytic system may be assumed to be the same before and after the membrane is made permeable.

Experimental Arrangements.

The method used for measuring the electric resistance of a tissue is the same as described in detail in my previous paper (7). The tissue is joined in series with a high resistance galvanometer, used ballistically, whose deflection gives the intensity of the current due to the potential difference between the two similar electrodes, immersed in the tissue, when connection is made through the tissue, the galvanometer, and the connecting wires and leads, which are of negligible resistance. For each observation the effective e.m.f. of the circuit, however caused, is determined by balancing it with an equal and opposite e.m.f. from a compound potentiometer. The current sensitivity of the galvanometer under the conditions of experiment is determined. From the deflection of the galvanometer and the readings of the compound potentiometer the resistance of the tissue at different temperatures was calculated.

For measuring the resistance of a plant tissue under varying temperature, certain precautions are necessary, particularly when the electrodes are thrust inside the tissue. Since the tissue must be kept in a moist chamber, if the electrodes are also in the chamber variation of temperature introduces an unknown change of contact resistance of the electrodes with the tissue. To overcome this, the main part of the organ is inserted in a thermal jacket with portions protruding at both ends, where electric contacts are made. These are kept at the room temperature, except for the conduction of heat from the tissue, which is negligible. This has the added advantage of enabling one to investigate the change of resistance at different temperatures after the tissue is killed by heat or chloroform. When the whole length of the tissue is killed a considerable amount of electrolyte escapes through the cut ends, but in this arrangement the living portions of the tissue outside the jacket prevent this and only a small portion escapes through the epidermis. The portion of the tissue between the electrodes, but not subjected to variation of temperature, was comparatively small; in case of stems and petioles 1 cm. out of 11 cm., and in *Aloe* leaf 2 cm. out of 14 cm. For greater accuracy in determination of resistance, variation correction proportional to the lengths can easily be made.

The Thermal Jackets.—For experiments with stems and petioles a double-walled cylinder of thin copper, about 10 cm. long, is used, the diameter of the outer cylinder being 10 cm. and that of the inner, which is open at both ends, 3 cm. The variation of temperature inside the cylinder is caused by a flow of water at different temperatures, from thermally and electrically insulated reservoirs of hot and cold water, through the annular space of the jacket. The two reservoirs are connected to the inlet tube of the jacket by a Y-tube. The stop-cocks of the reservoirs regulate alike the temperature of the water and its rate of flow through the annular space. By suitably adjusting the stop-cocks to make up for the loss or gain of heat by radiation and conduction, the temperature inside the cylinder can be maintained constant within 0·5° C. for nearly 2 hours. The jacket is thermally and electrically insulated by felt, cotton-wool, and asbestos rope, and is held horizontally by an insulated wooden stand. The inner cylinder is lined with a thick layer of moist blotting paper. Two rubber stoppers which fit the inner cylinder have two holes each and are split a little beyond the centre; the middle holes are for the tissue and one for thermometer, and the other, which is kept plugged with a rubber cork, allows the introduction of water for moistening the blotting paper. A shellacked glass rod which passes through the wooden clamp supports the electrodes.

For experiments with leaf of *Aloe perfoliata*, a much larger rectangular thermal jacket (figs. 1 and 2) with a thicker insulation is used, the jacket space being 12 cm. long, with a cross-section 10 × 6 cm., and silvered inside. To hold liquids inside the jacket (water for humidity, and chloroform for killing the enclosed portion of the organ) the lower inner sheet is converted into a shallow trough by two ridges, 1 cm. high, along the entire breadth of the jacket. Any liquid can be introduced inside the jacket through the funnel end of a ⅛-inch copper tube, which passes through the thermal insulation to one corner of the trough inside. On account of the shape of the *Aloe* leaf, ebonite sheets,

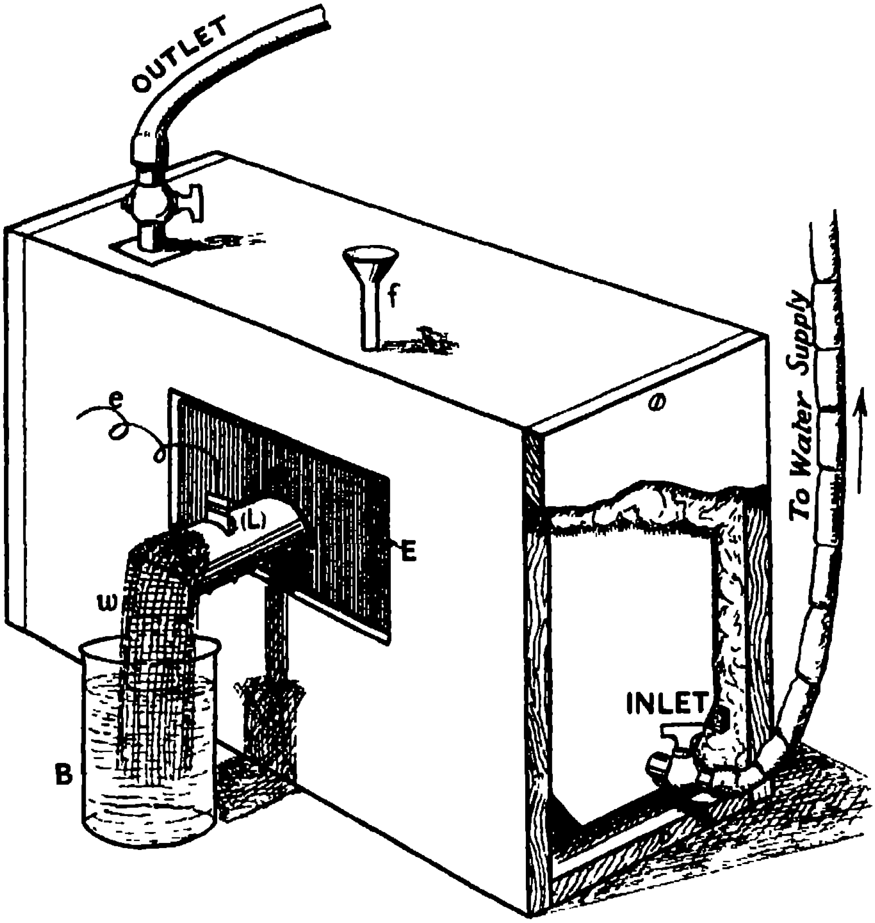

FIG. 1.—Diagram view of thermal jacket: L, leaf of *Aloe perfoliata*; *e*, electrode; E, ebonite sheet; black dot, thermometer hole; *f*, funnel for introducing liquid inside jacket space; B, beaker containing tap water; *w*, wet muslin.

11 × 7 cm. and 1 cm. thick, instead of rubber, are used to hold the leaf in the middle of the jacket space and close its two ends. The inner sides of these ebonite sheets are filed off to a depth of 5 mm. to fit the jacket ends, and the middle of these sheets are cut out in different curved shapes to allow the leaf to be inserted. In one of these sheets there is a hole for the insertion of the thermometer. Three layers of adhesive plaster of increasing width not only fix the ebonite sheets securely to the jacket, but also seal the edges adequately. The small gaps in the middle, between the ebonite and the leaf surface, are first plugged with strips of rubber tape and subsequently more securely sealed with adhesive plaster. With this arrangement it was possible to maintain the temperature of the jacket space constant for 5 hours by adequate manipulation of the reservoir stop-cocks for inflow of water.

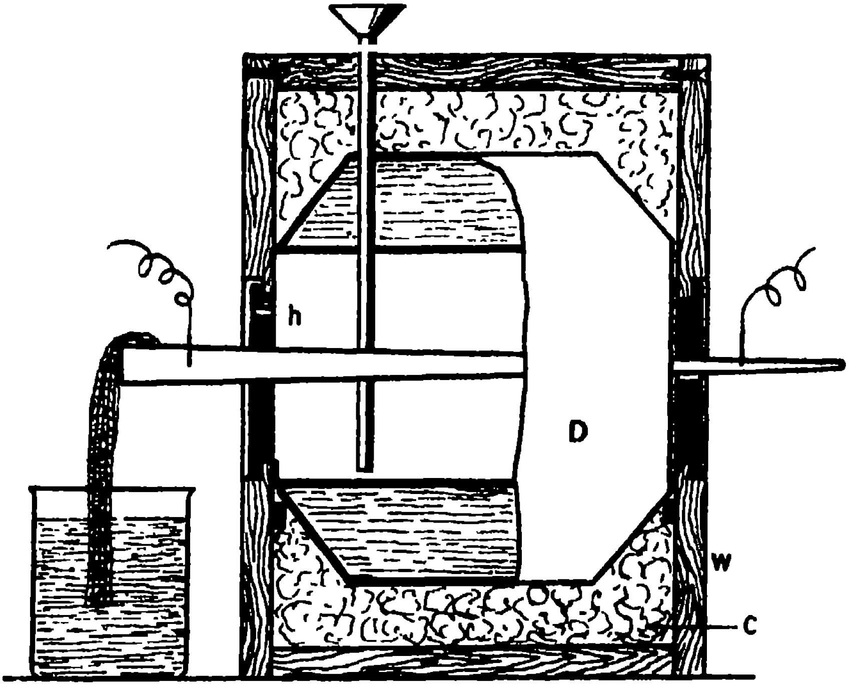

FIG. 2.—Section of jacket: D, double-walled metal jacket; C, cotton-wool; W, wood; *h*, thermometer hole.

Electrodes.—For stems and petioles, platinum wires, and for motile pulvinus of *Mimosa pudica*, gilt tinsels are used. For leaf of *Aloe perfoliata*, silver sheets, 10 × 15 mm. and 1 mm. thick, are used, and to overcome transference of any mechanical disturbance to the electrodes these are joined to the copper leads by strips of tinsel. The upper 5 mm. of these electrodes, as also the tinsel junction, are thoroughly insulated with shellac varnish, leaving an effective electrode area of about 1 cm.^{2}. It was found that sheets 1 mm. thick are better alike for handling and connection with the leaf. In the method employed for measuring resistance, any change of e.m.f. at the electrodes does not vitiate the results, therefore cheaper silver electrodes were used instead of platinum, with equal accuracy.

The Compound Potentiometer used in these observations consists of a Pye's rotating potentiometer and a half-metre bridge. The terminals of these were specially made of copper to eliminate thermo-electric complications.

The Galvanometer.—An Ayrton-Mather galvanometer, with a coil resistance of 6700 ohms and period 4·5 seconds, was used. Its sensitivity for 1 mm. deflection at 2 metres scale distance was $2 \cdot 2 \times 10^{-10}$ amp. To save time and patience while finding out by trial the potentiometer e.m.f. which exactly balances the tissue e.m.f., the galvanometer is made dead-beat, by using a double-poled mercury key, which interposes a damping resistance of 10,000 ohms; when the exact balance is secured the damping key is tilted off, and if the balance still remains undisturbed the potentiometer key is tilted off and the deflection of the galvanometer noted.

Experiments.

Experiments were first carried out with complex tissues, petioles and stems, to be described later. At the suggestion of Dr. F. F. Blackman homogeneous tissues were employed. In the leaf of *Aloe perfoliata* (commonly known in Bengal as *Ghritkumari* and highly valued for its medicinal properties), the central " aqueous tissue " is a homogeneous mass of large thin-walled vacuolate cells, and it is easy to find leaves with aqueous tissues 2 cm. thick.

Resistance of Leaf of Aloe perfoliata *at Different Temperatures in the Living and Dead Conditions.*

As far as possible, leaves of similar age and dimensions, from potted specimens of *Aloe perfoliata*, were used for experimental material. A length of 35 to 40 cm. of a leaf was isolated and inserted into the rectangular thermal jacket; only about 12 cm. of the leaf enclosed in the jacket was subjected to variation of temperature, the rest projecting out from both ends of the jacket. The ends of the leaf were cut afresh and covered with a thick layer of wet muslin dipping into insulated beakers of tap water. After sealing the jacket space with adhesive plaster in the manner already described, about 20 c.c. of water was introduced inside the jacket trough through the projecting funnel on top. About 1 cm. beyond the enclosed portion of the leaf, fine incisions were made on both sides through drops of water with a Gillette blade and the electrodes were thrust in through these incisions to a depth of 13 to 14 mm., so that the effective electrode surface was in contact only with the central homogeneous aqueous tissue of the leaf, the green heterogeneous layer, about 2 mm. in depth, being in contact with the insulated portion of the electrodes. A thick layer of kaolin-glycerin-saline paste was applied all round the contact and covered with three to four coatings of thin solution of pure shellac. The thermometer was inserted inside the jacket through the hole in the ebonite.

After attempting several different arrangements for maintaining the electrodic resistance constant for the long period of observation, it was found that the simple technique described above was the most reliable. A leaf of *Aloe* thus mounted could be used for experimental observation for two or three days in succession. The temperature of the jacket was changed very slowly, 10° C. in the course of half an hour. When the enclosed portion of the tissue had to be killed at different temperatures, about 30 c.c. of chloroform (10 c.c. at intervals of 15 minutes) was poured through the funnel into the trough inside the jacket. By inserting a thermometer through the middle of the leaf along its length, preliminary observations were taken to find the time it took the leaf to attain the temperature of the jacket. This was found to vary from 45 minutes to an hour, therefore all resistance measurements were taken an hour after the jacket attained a steady temperature.

Observations.—(1) After the leaf was mounted in the jacket and electric connections made, it was allowed to recover overnight. The next morning its resistance at 20° C. was observed till a steady value was found, which was generally 12 to 14 hours after mounting. The temperature of the jacket was then raised to 30° C. in the course of half an hour, and maintained constant within 0·5° C. for 5 hours. Resistance measurements at intervals of 15 minutes were taken an hour after the temperature of the jacket had reached 30° C. Exactly similar procedure was followed for resistance measurements at 40° C., after which the temperature of the jacket was slowly lowered and the resistance of the leaf measured the next day at the same temperatures. Over 30 specimens were experimented upon, some at Almora in the Himalayas with the help of Miss H. B. Brewster, and I have repeated one series of observations in Prof. Blackman's laboratory in London. The nature of the resistance changes has invariably been the same.

At the suggestion of Prof. Blackman observations were taken to confirm that under the conditions of the experimental arrangement the main path of conduction was through the central homogeneous tissues, and not through the outer heterogeneous layer of tissues. It was found that the specific resistance of the outer green tissue is certainly not less than that of the homogeneous central tissue, and as the cross-section of the central tissue is so much greater than the superficial green tissue, the main part of the current must certainly be carried by the central tissue. The observed changes of resistance therefore indicate mainly the changes of the central colourless aqueous tissue.

In Table I a typical experiment is given in detail.

Table I.—Showing Resistance (ohms) of Leaf of *Aloe perfoliata* at Different Temperatures: (1) the First Day, and (2) the same Leaf Next Day.

Time in minutes.	(1) Temperature.			(2) Temperature.		
	20° C.	30° C.	40° C.	20° C.	30° C.	40° C.
—	96,384	74,828	68,100	95,964	73,992	67,298
15	96,364	74,719	68,094	95,070	73,808	67,202
30	96,374	74,724	68,223	95,082	73,800	67,201
45	96,388	74,680	68,198	95,071	73,709	67,299
60	96,380	74,633	68,211	95,072	73,681	67,286
75		74,510	68,284		73,504	67,308
90		74,400	68,310		73,461	67,421
105		74,612	68,416		75,389	67,388
120		74,508	68,341		73,470	67,395
135		74,400	68,411		73,385	67,573
150		74,358	68,579		73,321	67,505
165		74,331	68,584		73,281	67,498
180		74,310	68,578		73,232	67,599
195		74,300	68,592		73,211	67,560
210		74,259	68,587		73,202	67,560
225		74,258	68,591		73,201	67,558
240		74,259	68,580		73,202	67,600

It will be seen from the above table that (i) the resistance of the leaf at 30° C. attains a fairly steady value in 3 hours, and at 40° C. within an hour. (ii) *the diminution of resistance per ° C. between temperatures* 20° *to* 30° *C. is* 2·3 *per cent., while at higher temperatures from* 30° *to* 40° *C. the diminution of resistance is far less, only* 0·7 *per cent.*; (iii) with time the drift of variation of resistance at 30° C. is towards a decrease, and at 40° C. towards an increase; (iv) the slight increase of resistance found after a time at 40° C. is not due to an increase in the contact resistance, since the resistance of the leaf at each temperature was less than on the previous day; and (v) the resistance change observed up to 40° C. is reversible, since almost identical variation of resistance with rising temperature was observed in the same leaf the next day.

In the 30 specimens examined the resistance of the leaf at 30° C. was found to decrease with time, varying from 1 per cent. to 0·5 per cent. in the course of 4 hours; for the same period resistance at 40° C., increase from 0·7 per cent. to 0·4 per cent.; when the leaf was cooled down slowly to 20° C., its resistance was found to be less than the starting value. The decrease of resistance observed at 30° C., as compared with 20° C., varied between 2·7 per cent. and 1·8 per cent. per degree; while for temperature 30° and 40° C., it was found to vary from 1 per cent. to 0·4 per cent.

Preliminary experiments were carried out to observe the variation of resistance of a leaf previously killed by chloroform vapour. A wide-mouthed Dewar flask was used as a chloroform chamber. A portion of a leaf about 25 cm. long, with a small beaker just below it, was suspended by strings from a hook fixed to the lower end of the flask cork. After pouring about 40 c.c. of chloroform on the cotton-wool at the bottom of the flask, it was closed by the cork with the leaf and the beaker hanging from its bottom. After 12 hours the cork was opened, and the discoloured leaf wiped dry and smeared with vaseline and mounted in the jacket. The specific resistance of the exudate which collected in the beaker was found to be 96·6 ohms. The resistance of the dead leaf was found to increase steadily; in course of 24 hours it increased by 48 per cent. This was mainly due to the increase of resistance at the electrodes and escape of electrolytes through the cut ends, as shown by the fact that when only the portion of leaf enclosed in the jacket was killed by chloroform and the electrodes were inserted in the projecting living portion of the leaf, the increase of resistance in course of 24 hours was from 3 to 4 per cent. only. No further experiments with killed leaves, otherwise than in the thermal jacket, were carried out.

Observations.—(2) The leaves were mounted in the jacket in the usual way, but the electrodes were inserted 2 cm. (instead of 1 cm. as in the previous experiment) beyond the enclosed portion of the leaf, so as to leave room for the electrodes to be inserted again in the living portion of the tissue after killing the central enclosed portion by chloroform. The resistance of 15 leaves of *Aloe* at temperatures 20°, 25°, 30°, 35° and 40° C. was observed for an hour after the leaf attained the temperature of the jacket. A deduction of 0·7 per cent. in the observed resistance was made for the temperatures 20° to 35° C. and an addition of 0·5 per cent. for 40° C., as correction for time-effect shown in the previous experiments. Some of the leaves were killed at 30° C. and others at 40° C. by introducing chloroform into the jacket. The marked diminution of resistance on death was measured till it attained a steady value at those temperatures. It takes much longer to kill it at 30° C. than at 40° C.

The temperature of the jacket was cooled down and the leaf taken out of the jacket, wiped dry, and the chloroformed portion of the leaf smeared with vaseline to check the escape of electrolytes through the epidermis. The leaf was mounted again, the chloroformed portion being enclosed in the jacket and the electrodes inserted in new positions, about 1 cm. beyond the killed portion, and the resistance measured at temperatures 20°, 30° and 40° C. in the usual way. The period of observation was purposely shortened, as in the killed tissue the escape of electrolytes through the epidermis increases with time and temperature in spite of the vaseline coating. The diminution of resistance of *Aloe* leaf on being killed by chloroform was found to vary from 250 to 300 per cent.

The detailed observation of resistance of a leaf, before and after it was killed by chloroform at 40° C., is given in Table II. In Table III is given the percentage variation of resistance of five leaves. From these it will be seen that in dead leaves the diminution of resistance with rise of temperature is fairly constant, if we consider the electrolytes which escape through the epidermis, the specific resistance of the exudate being very low (100 ohms). If we take 1·6 per cent. decrease of resistance for a rise of 1° C. as the purely *physical effect* on the electrolytes of the tissue, then between temperatures 20° to 30° C. the diminution of resistance in a living *Aloe* leaf is more than could be thus accounted for. Between 30° to 35° C. it is very nearly the same, while between 35° to 40° C. the decrease of resistance is decidedly less than that due to the physical effect of rise of temperature.

Table II.—Showing Resistance of *Aloe* Leaf at Different Temperatures, both in the Living and after being Killed by Chloroform.

Temperature. °C.	Resistance in ohms.	
	Living.	Dead.
20	98,234	24,864
25	87,426	
30	78,684	20,622
35	73,175	
40	70,264	17,529

Table III.—Showing Percentage Variation of Resistance per ° C. at Different Temperatures for *Aloe* Leaf, before and after Killing by Chloroform Vapour.

Specimen, living.	Temperature range.			
	20°-25° C.	25°-30° C.	30°-35° C.	35°-40° C.
	Per cent.	Per cent.	Per cent.	Per cent.
I	2·2	2·0	1·4	0·8
II	2·6	2·3	1·6	0·5
III	2·5	2·4	1·5	0·4
IV	2·1	2·0	1·5	0·3
V	2·7	2·4	1·7	0·2

Specimen, dead.	Temperature range.	
	20°-30° C.	30°-40° C.
	Per cent.	Per cent.
I	1·7	1·4
II	1·3	1·2
III	1·5	1·3
IV	1·6	1·5
V	1·7	1·5

Experiments with the Stem of Bassela alba *and the Petiole of* Helianthus annuus.

Experiments similar to those described for the leaf of *Aloe* were carried out with the stem of *Bassela alba* and the petiole of *Helianthus annuus*. In fact, as already stated, these were the first experiments in which the peculiar change of electric resistance with rise of temperature was observed. The experimental technique was similar to that described in the previous set of experiments, only the temperature of the jacket was raised by steps of 5° C. in 10 minutes and was kept at that temperature from 15 to 20 minutes according to the thickness of the tissue, so as to attain the temperature of the jacket. The mean value of three successive observations at intervals of 5 minutes was taken as the resistance at that temperature. This procedure was repeated for each gradual rise of 5° C. till a marked diminution of resistance was observed, which was taken as the death-temperature, and was found generally between 55° to 62° C. according to the condition of the tissue. After this the jacket was cooled down to the original starting temperature, kept at that temperature for an hour, and observations taken with rising temperature by steps of 10° C. Detailed observations are not given here, since they are similar to those found with the more trustworthy results obtained with the homogeneous internal tissue of the leaf of *Aloe*.

Observations.—(3) Over 20 experiments with each of these organs were carried out, mostly for the temperature range 30° to 40° C. Invariably it was found that the diminution of resistance from 30° to 35° C. was considerably greater than that for 35° to 40° C. The percentage diminution at the lower range was on an average 1·8 per cent. per degree, and 0·5 to 0·1 for the higher range. In some cases the resistance at 35° C. was the same as at 40° C., while in a few cases it was found to be greater. Up to 40° to 45° C. the temperature effect was found to be reversible. For the range 40° to 55° C. hardly any change of resistance could be observed, till a temperature from 55° to 62° C. is reached, when a marked diminution of resistance as also a reversal of the direction of the tissue e m.f. occurs, as has been previously observed by Bose (4). The resistance of the killed tissue at different temperatures was found to show a steady diminution throughout the entire range, except for the exosmosis of electrolytes through the epidermis.

Experiments with the Pulvinus of Mimosa pudica.

All the experiments were carried out with the petiole-pulvinus preparation of *Mimosa pudica* (5). To avoid variable light conditions observations were taken in a dark humid chamber. Fig. 3 shows the experimental arrangement. Along with resistance determination the turgor variation of the pulvinus, as indicated by the movement of the magnifying lever attached to the petiole, was noted at the same time. The thin three-ply board which covers the thermal chamber, and from which the petiole-pulvinus preparation and the electrodes are suspended without touching the chamber, is previously heated and thoroughly shellacked. The cover rests on four porcelain insulators on top of the chamber, this alike for insulation and ventilation. The thermal chamber is both electrically and thermally insulated. The temperature variation of the chamber is effected by the method already described.

Electrodes.—The tinsel electrodes used in these experiments not only permit free movement of the leaf, but greater surface of contact could be obtained by winding the tinsel two or three times round the petiole without affecting the motility of the pulvinus. After the tinsel is securely tied it is covered with a layer of kaolin-saline-glycerin paste by a fine camel-hair brush, and the whole contact is covered with shellac solution for preventing evaporation and

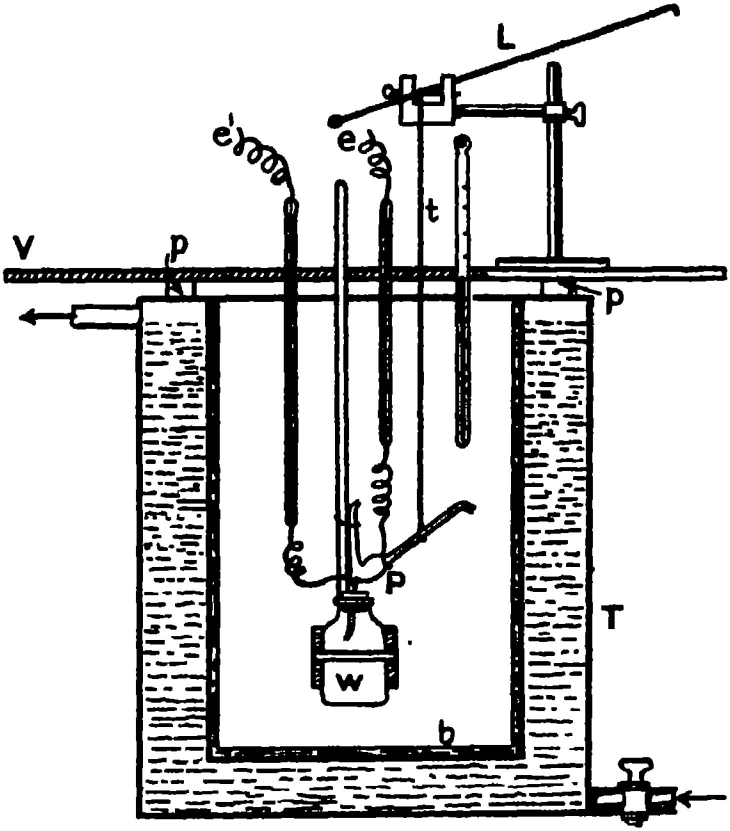

FIG. 3.—Diagram of petiole-pulvinus-preparation of *Mimosa pudica* in thermal chamber: T, double-walled thermal chamber lined inside with moist blotting-paper, *b*; V, three-ply board resting on porcelain insulators, *p*; P, petiole-pulvinus-preparation mounted in flat phial, W, containing tap water; *e*, *e'*, electrodes for measuring resistance; *t*, paraffined silk thread connecting petiole to lever, L. Arrow indicates direction of water flow.

condensation of, from, or at, the contact. A fine silk thread soaked in molten paraffin is tied to the petiole, the other end being attached to the short arm of the lever through a hole in the cover. The variation movement of the petiole was measured by the excursion of the long arm of a lever of Bose-type, constructed by Mr. N. N. Sen Gupta of the Presidency College. A millimetre-squared paper pasted on to a plane glass plate, placed at right angles just clear of the bent tip of the long arm of the lever, gave a very accurate measurement of the movement of the petiole.

Observations.—(4) Over 15 different specimens of *Mimosa* pulvinus were experimented upon and it was found that the change of resistance of the pulvinus at different temperatures was similar to that found in the *Aloe* leaf, the petiole of *Helianthus annuus* and the stem of *Bassela alba*, *i.e.*, the diminution of resistance was greater in the living condition from 25° to 35° C. and the corresponding movement of the petiole was downwards (fig. 4), indicative of diminution of turgor, while beyond 37° C. the diminution of resistance was hardly noticeable, the concomitant movement of the petiole being upwards and so indicative of increase of turgor. Between 55° to 60° C. a marked diminution of resistance and a sudden down-movement of the petiole was observed.

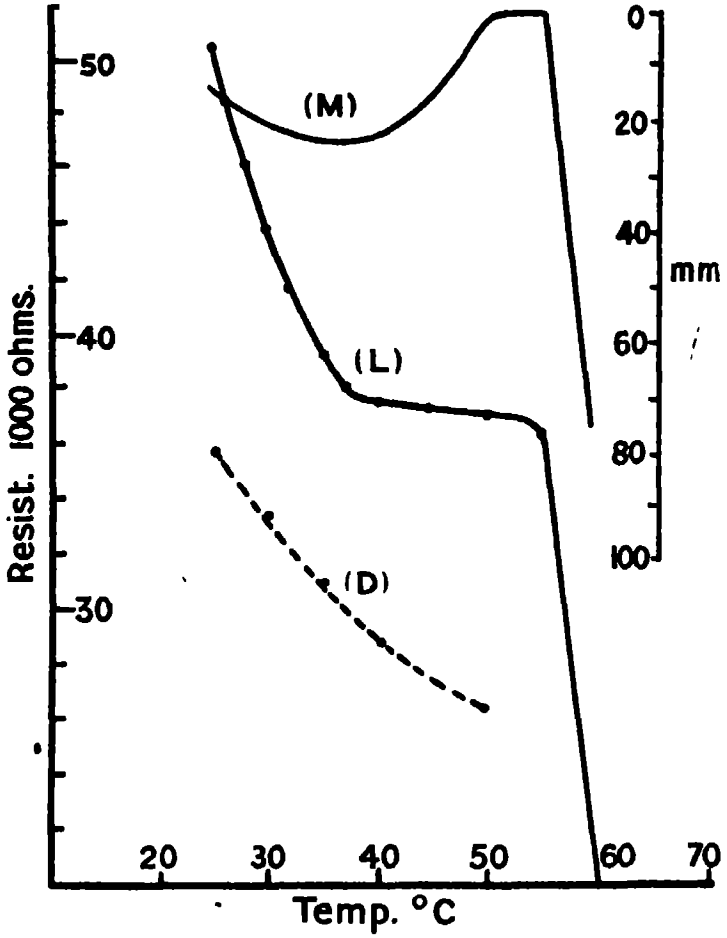

FIG. 4.—Curves showing resistance at different temperatures of *Mimosa* pulvinus: L, living; D, dead (dotted), and M, mechanical movement of leaf.

In Table IV the detailed results of one experiment are given. In fig. 4 curves constructed by plotting resistance against temperature, as also the mechanical movement of the petiole in millimetres, are given. The resistance of the dead pulvinus was also observed, see the dotted curve in fig. 4. The lower percentage diminution of resistance of the dead pulvinus is due to the escape of electrolytes into the water in the small bottle in which the petiole-pulvinus preparation was mounted. The changes up to 45° C. have been found to be reversible.

Table IV.—Showing Resistance of *Mimosa* Pulvinus at Different Temperatures, both in the Living and Dead Conditions.

Temperature °C.	Resistance in 100 ohms.	
	Living.	Dead.
25	508	359
28	465	
30	441	337
32	419	
35	394	310
37	382	
40	378	290
45	378	
50	372	265
55	368	
60	201	

Conclusions.

The experiments described show that for moderate temperature rise—20° to 30° C., in *Aloe* leaf and *Bassela* stem, and 20° to 35° C. in *Helianthus* petiole and *Mimosa* pulvinus—there is a steady diminution of electric resistance; for temperatures above this (30° to 35° C. in *Aloe* and *Bassela*, and 35° to 40° C. in *Helianthus* and *Mimosa*) the diminution is much less; above these temperatures the decrease of resistance becomes still slower or may not occur at all; with still further rise of temperature the tissues are killed and there is an enormous decrease of resistance.

When these changes in a living organ are compared with those in the same organ after death, it is found that the diminution of resistance in the dead tissue is very nearly uniform, as is to be expected. Furthermore, it is seen that for moderate rise the decrease of resistance is greater in the living tissue than in the dead; with higher temperature the fall in the resistance is about the same in the living and in the dead tissues; at a somewhat higher temperature (beyond 35° and 40° C.) the fall is far less in the living tissue. This condition is continued until with rising temperature (about 60° C.) the cells are killed and a large decrease of resistance ensues.

The dead tissue exhibits the purely physical effect of temperature in decreasing resistance by increasing the mobility of the ions. The fact that there are such marked differences between the responses to temperature of living and dead tissues indicates that the permeabilities of the plasma membrane of the cells to ions are affected by temperature changes. The interesting result is thus brought out that the effect of increasing temperature is not a steady increase of permeability, as might be expected. The effect between 20° to 30°–35° C. is an increase of permeability, then the rate of increase falls off, and the permeability may not change at all with rise of temperature. With further rise (beyond 35° and 40° C.) the permeability decreases, until finally with the death of the cell the resistance of the plasma membrane breaks down and the electric resistance decreases enormously.

Summary.

The effect of temperature on the permeability of plant cells to ions has been investigated by measuring the electric resistance to a direct current of various plant organs, on the assumption that such changes of resistance indicate a change of permeability of the plasma membrane to ions.

By using the method of measurement in which the plant itself supplied the e.m.f. producing the current which is measured, the electric resistance of the homogeneous aqueous tissue of the leaf of *Aloe perfoliata*, the stem of *Bassela alba*, the petiole of *Helianthus annuus*, and the pulvinus of *Mimosa pudica* at different temperatures has been observed, both in the living condition and after killing by chloroform or heat.

These different observations show that the permeability of the plasma membrane to ions (i) increases with rising temperature, *i.e.*, from 20° to 30° C. in *Aloe* leaf and *Bassela* stem, and from 20° to 35° C. in *Helianthus* petiole and *Mimosa* pulvinus; (ii) from 30° to 35° C. in *Aloe* and *Bassela* and 35° to 40°C., in *Helianthus* and *Mimosa* there is hardly any increase; (iii) beyond 35° or 40° C. and up to the lethal temperature the permeability decreases, the changes observed up to 40° or 45° C. being reversible; (iv) at the lethal temperature the plasma membrane becomes highly permeable, a change which is, of course, irreversible.

I take this opportunity to acknowledge the financial help received from Mr. and Mrs. L. K. Elmhirst, and Mr. Glen Overton, which has made it possible to start a small laboratory, to Prof. A. V. Hill for his unfailing help and encouragement; to Dr. F. F. Blackman for his suggestions; and particularly to Prof. V. H. Blackman for his help and criticism. I have also to acknowledge the help of the Government Grants Committee of the Royal Society in purchasing some of the apparatus used in this investigation.

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The Vital Staining of Normal and Malignant Cells. I.—*Vital Staining with Trypan Blue, and the Cytoplasmic Inclusions of Liver and Kidney Cells.*

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1. *Introduction.*

Although *intra-vitam* staining has now become a routine method in histology, the application of this technique to the special problems of the cell is comparatively recent. Vital staining has been fully discussed by von Mollendorff (1920, '21, '23, '26). Full references to the literature, and the main results of vital staining, are to be found in his valuable reviews, in which he lays down the fundamental principles of the subject.

2. *Principles of Vital Staining.*

There are two chief methods of vital staining. Either the dye can be injected into the body of the living animal—*intra-vitam* staining; or fragments of living tissue can be removed from the animal and immersed in dilute solutions of the dye—supra-vital staining. With basic dyes the latter method is usually employed owing to the difficulty of retaining the dye in fixed preparations. Basic dyes are also used *intra vitam*, but most are more toxic than acid dyes. Whichever method is employed, with basic dyes, in general, there is an actual staining of pre-formed structures. Most investigators are in agreement that it is only non-living granules, or droplets, within the cytoplasm that are stained with basic dyes.

Vital staining with acid dyes is usually carried out by injecting a dilute solution of a relatively non-toxic dye into the living animal, either subcutaneously or intravenously, or else intraperitoneally. The rate of diffusibility of dyes determines to a great extent their efficacy for this purpose. Following injection of an appropriate stain, droplets make their appearance in certain of the cells. Those of the reticulo-endothelial system show special propensities for taking up the dye, with which their cytoplasm becomes rapidly filled. Many of the cells of the animal body have not been found to stain, yet the trend of recent research has been to extend the list of cells which do stain with acid dyes.

It is generally agreed that an acid dye, such as trypan blue, which enters the living cell, does so in the form of a dilute colloidal solution, and then becomes collected into droplets in certain parts of the cell. This is how von Möllendorff (1921) describes the process :—

> "Zu verschiedenen Zeiten der Farbstoffausbreitung innerhalb des Tierkorpers kann man verfolgen, wie innerhalb der Zellen die Granula an Zahl, Grosse und Farbdichte allmählich zunehmen; dabei erhält man den Eindruck, dass zuerst die Granula den Farbstoff in einer sich allmählich konzentrierenden Losung enthalten. Sie sehen durchscheinend aus. Erst bei weiterer Farbstoffzufuhr wird der Tropfeninhalt inhomogen, manchmal sind tanzende Körnchen innerhalb der Vakuolen zu beobachten, bis eine vollständige Ausfallung des Farbstoffes in dem Tropfchen zustandekommt, wodurch ein Granulum die maximale Farbstoffmenge aufgenommen hat" (p. 118).

The essential features of vital staining with acid dyes are, therefore,

(1) It is a purely physical process.

(2) In the living cell there is no staining of pre-formed structures : the dye droplets are new formations.

3. *Vital Staining with Acid Dyes and the Cell Organs.*

If one injects 1 c.c. of a 1 per cent. solution of trypan blue subcutaneously into a mouse, a considerable accumulation of dye can be seen in the cells of the convoluted tubules of the kidney twenty-four hours later. Comparison of a frozen section of such a kidney with mitochondrial and Golgi apparatus preparations will show—

(1) That there is no obviously direct relationship between the mitochondria and the dye droplets ;

(2) That there is remarkable similarity between the arrangement and position of the dye droplets, and the form and distribution of the Golgi apparatus.

Jasswoin (1925) was the first to point out that trypan-blue droplets are formed in kidney cells in that area of the cytoplasm where the Golgi apparatus is situated. He showed that in cells of the convoluted tubules of Amphibians the position of the Golgi apparatus varies in different physiological conditions, and in each case there is complete agreement in the position of the apparatus and the dye droplets. By the methods Jasswoin employed he was not able to obtain either mitochondria or Golgi apparatus in the same preparation as the dye droplets, although in some of his Golgi apparatus preparations he found the latter were represented by brown granules.

It has been established by recent research work that in gland cells the secretion granules or droplets originate in the cytoplasm in close relationship with the Golgi apparatus. To Nassonov is due the credit for having drawn attention to the essential similarity in the way in which secretion droplets arise in gland cells, and droplets of dye are formed in vital staining with acid dyes. Nassonov (1926) investigated specially the relationship between trypan-blue droplets and the Golgi apparatus in the kidney and liver cells of several vertebrates. He found complete agreement between the position and arrangement of dye droplets and of the Golgi apparatus in the early stages of *intra-vitam* staining. Furthermore, just as secretion granules of gland cells originate within, or in contact with, the Golgi apparatus, and later move away towards the outer wall of the cell, so do the trypan-blue droplets in the cells of the convoluted tubules. The same relationship between apparatus and dye was also found to hold in liver cells. These observations led Nassonov to attribute a definite physiological function to the Golgi apparatus. He says that—

> " In den normalen Zellen dieses oder jenes Secret im Plasma chemisch vorbereitet wird, während sich die Tätigkeit des Golgi-Apparats auf die elektive Konzentration dieses Secrets und auf die Bildung von Granula oder Vacuolen beschränkt " (p. 500).

Nassonov's paper was published at the same time as a paper by Cramer and the present writer (1926) on the cellular mechanism of bile secretion. We drew attention in this communication to the relation between the Golgi apparatus of the liver cells and the intercellular bile capillaries. From the study of the Golgi apparatus of liver cells under various physiological conditions, we concluded that certain constituents of the bile were formed in relationship with the apparatus in the same way as secretion granules are in gland cells.

The same year a paper was published by Makarov (1926) on this subject. He found in a large number of vertebrates that the Golgi apparatus of the liver cells was arranged around the intercellular bile capillaries. Further, on injecting trypan blue into the animals, dye droplets made their appearance in the region of the Golgi apparatus.

The latest contribution to this problem has been made by Glasunow (1928). He injected trypan blue into guinea pigs over relatively long intervals of time. By this method he was able to stain cells which previously had been considered unstainable intra-vitally. He also found that after a short period of injecting the dye, it appeared in certain of the cells, such as those of the liver and kidney, in the form of granules. After longer periods of injecting, such granules anastomosed to form filaments, and finally assumed the typical reticulate form of the Golgi apparatus.

The position of this problem up to the present time is, therefore,

(1) Injection of trypan blue for relatively prolonged periods brings about a deposition of the dye in certain cells identical in appearance with the Golgi apparatus.

(2) Injection of trypan blue for shorter periods results in a deposition of the dye as droplets in definite areas of many cells The general arrangement and distribution of such droplets are identical with the Golgi apparatus.

The next step in the solution of this problem would therefore seem to be to attempt to demonstrate both Golgi apparatus and dye droplets in the same preparation. The technique by which this can be accomplished, and the results obtained by it, are described in this paper.

4. *Cytological Technique for Intra-Vitam Staining with Trypan Blue.*

Following the advice of von Mollendorff, I have injected mice with 1 per cent. or 0·5 per cent. trypan blue. The 0·5 per cent. solution has been found to be more satisfactory than a 1 per cent. solution with experiments conducted over relatively long periods. In my latter experiments, half a cubic centimetre of a

0·5 per cent. solution of the dye was injected on alternate days over various lengths of time. After three consecutive injections, an interval of two days was allowed before the next injection. All injections were made subcutaneously. It is desirable to inject as deeply as possible, and, as von Möllendorff has recommended, to employ gentle massage so as to spread the dye as much as possible under the skin.

The cells of the convoluted tubules of the kidney are deeply stained after one or two injections. Most of our observations have been carried out on the cells of the liver and kidney of mice which have received five or six injections over a period of about a fortnight. Tissues have been fixed from 6 to 24 hours after the last injection.

The cytological technique to be employed with tissues stained *intra vitam* depends upon the structure it is desired to demonstrate in addition to the dye droplets. Material can either be fixed in formalin and then cut frozen, or can be embedded in paraffin and sections cut in the usual way. Von Möllendorff recommends as the best fixatives with trypan-blue stained material, firstly, formalin, 5 to 10 per cent.; secondly, concentrated sublimate solution. He also adds that the potassium bichromate method of Altmann's for mitochondria can be used.

The technique we have employed is as follows :—

(i) *For Dye Droplets and Nuclear Structures. Frozen Section.*—Tissues have been fixed either in 40 per cent. formalin for two or three hours, or in 10 per cent. formalin in normal saline overnight. Sections cut with a CO_2 freezing microtome were stained with neutral red, as previously described for modified Kopsch sections.

Paraffin Section.—Material has been fixed in formol-sublimate (8·5 parts of saturated corrosive sublimate to 1 part of formol). It is desirable to bring the fixed material as rapidly as possible through the alcohols. Neutral red has been employed for counterstaining the sections.

(ii) *For Dye Droplets and Mitochondria.*—Frozen sections of tissues fixed in formol, and stained with Hollande's iron carmine, have given good results with the liver and kidney. Sections have been stained a minute, or less, in the carmine solution, rinsed, then blackened with iron alum and rapidly differentiated. After washing, they have been dipped in pyridin, then washed in running tap water before mounting. In successful preparations the mitochondria are dark brown, and the trypan-blue droplets bluish black to pale blue.

(iii) *For Dye Droplets and Golgi Apparatus.*—Previous investigators have failed to obtain the Golgi apparatus and the dye in the same preparation. Both Jasswoin and Nassonov worked with the Kolatschev method. Nassonov points out that he based his conclusions upon a comparison of vitally stained preparations with sections demonstrating only the Golgi apparatus, "weil alle meine Mühe die Vitalfärbung an imprägnierten Präparaten zu erhalten, keine positive Resultate ergaben" (p. 481).

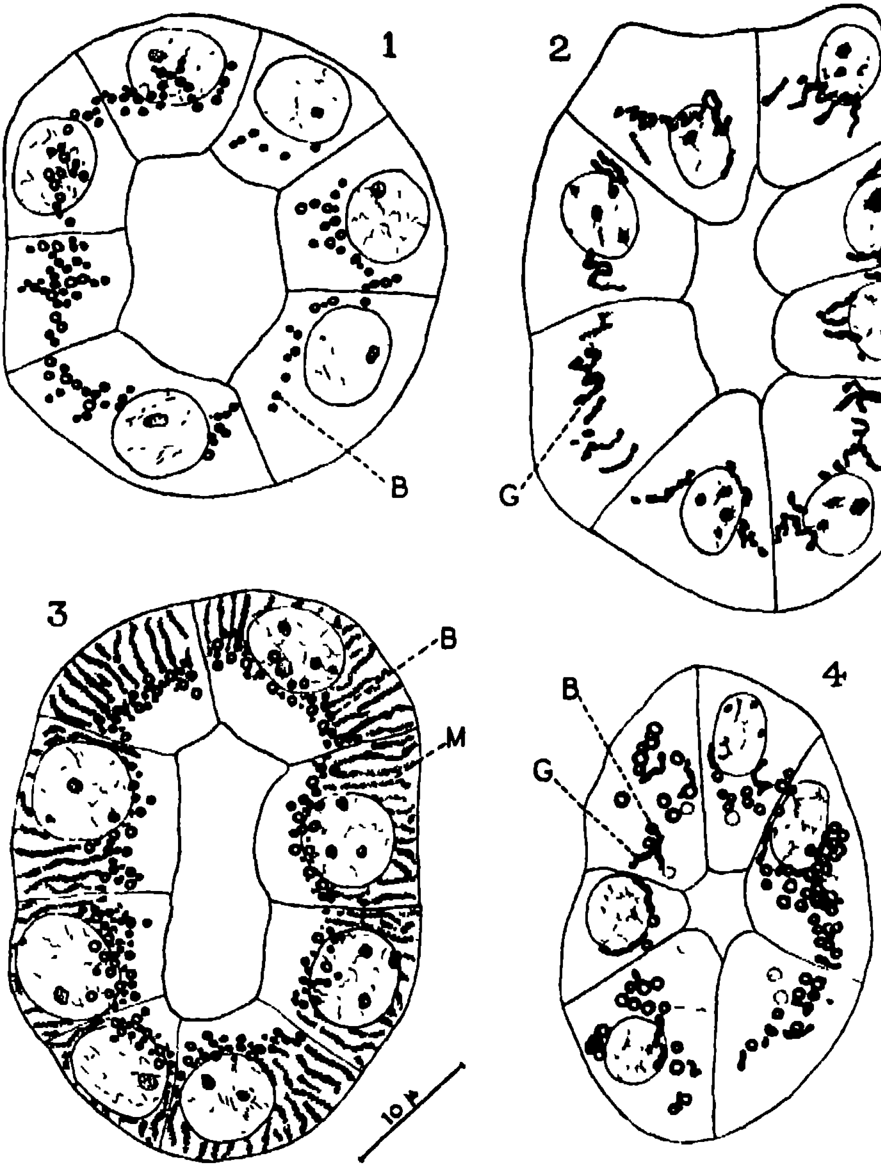
Since, according to von Mollendorff, corrosive sublimate is one of the best fixatives for trypan-blue stained tissues, it seemed probable that by employing the modified Kopsch method I have used previously, successful preparations might be obtained. After having tried a number of variations of this technique, the following has given the most satisfactory results :—

Very small pieces of tissue were cut up with a safety-razor blade and fixed overnight in corrosive-osmic solution (equal parts of 6 per cent. corrosive sublimate and 2 per cent. osmic acid). After fixation the fragments were washed for one-half to one hour in distilled water, which was repeatedly changed. The material was then transferred to 2 per cent. osmic acid, and kept in an incubator at 35° C. for 2½ days. At the end of this time the osmic acid was poured off and the fragments of tissue were washed with distilled water at 35° C. The water was continually changed until no smell of osmic acid remained ; then the material, still in distilled water, was put back in the incubator for another 24 hours. Finally, it was brought up through the alcohols during the course of seven or eight hours, left overnight in soft wax (45° C. melting point), and embedded in hard wax the next morning.

Sections were cut 3 μ in thickness, and either mounted directly in balsam, or else counterstained lightly with neutral red. This method has given excellent results with the kidney. In the liver cells, however, there is a less intense intra-vital staining with trypan blue, and the dye droplets are not so clear. It was, therefore, found convenient to counterstain the trypan-blue droplets with the basic dye, neutral red. This method has also been employed with sections of the kidney.

5. *Relation of the Dye Droplets to the Cytoplasmic Structures in the Kidney.*

The general arrangement of the dye droplets in cells of the convoluted tubules of the kidney, 24 hours after subcutaneous injection of 1 c.c. of 1 per cent. trypan blue, is shown in fig. 1. In the next figure is represented the arrangement of the Golgi apparatus (G) in the cells of a convoluted tubule of



Figs. 1–4.—Cells of the convoluted tubules of the mouse—all, except fig. 2, stained intra-vitally with trypan blue.

Fig. 1.—Typical arrangement of trypan-blue droplets (B) 24 hours after a subcutaneous injection of 1 c.c. of 1 per cent. trypan blue.

Fig. 2.—Golgi apparatus (G) in cells of a normal mouse.

Fig. 3.—Mitochondria (M) and dye droplets (B).

Fig. 4.—Golgi apparatus and dye droplets.

(Protocol of experiment of which figs. 3 and 4 represent the result: 1st day—0·5 c.c. of 1 per cent. trypan blue injected. 4th day—0·5 c.c. of 1 per cent. trypan blue injected. 7th day—0·5 c.c. of 1 per cent. trypan blue injected. 8th day—tissues fixed.)

a normal mouse. These two figures illustrate the essential features of vital staining of such cells with trypan blue, namely :—

(i) That there is no diffuse staining of the living cell ;

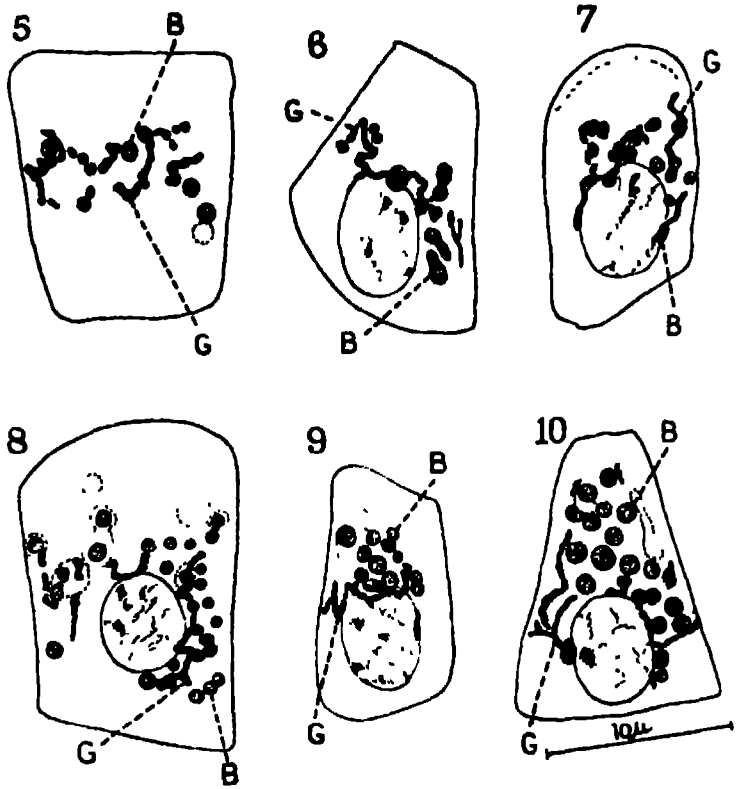
(ii) That the dye accumulates in the cell as droplets in a definite area of the cytoplasm ; and

(iii) That the general arrangement of the dye droplets coincides with that of the Golgi apparatus.

It was by comparison of such figures that Nassonov arrived at his conclusion as to the function of the Golgi apparatus. There have been workers who have attributed to the mitochondria a rôle in the staining process. Such a view has not been sustained by recent researches. Fig. 3 shows the mitochondria (M) and dye (B) in a frozen section stained by Hollande's carmine method. The mitochondria (M) present the typical filamentous form of such cells. At the inner extremities of the long filaments, granular mitochondria occur. They appear to be mixed up with the dye droplets. It is impossible to tell whether such granular mitochondria may take up the dye. One can, however, definitely conclude that there is no accumulation of the dye by the mitochondria, as a whole, in the living cell.

The intimate relationship existing between the dye and the Golgi apparatus is shown in fig. 4, which represents a section of a tubule from the same animal as fig. 3. Since by the method already described both dye and Golgi apparatus have been demonstrated in the same preparation, it has been possible to figure in detail the relationship between the two This is shown in figs. 5 to 10, and these figures serve as a confirmation of Nassonov's view. They show that the dye droplets (B) arise in association with the Golgi apparatus (G). and as with secretion granules in gland cells, the formed droplets move out away from the apparatus into the cytoplasm. Figs. 5 to 10 are arranged as a progressive series to illustrate what may be regarded as successive stages in the formation and accumulation of dye droplets (B) within the cell.

It has been noticed occasionally in examining sections that part of the apparatus has reduced the osmic acid and appears black, while other parts seemed to be stained blue. This is shown in figs. 6 and 7. Such an appearance is probably due to the dye accumulating at the surface of the apparatus and opposing a barrier to the penetration of the osmic acid.



Figs. 5–10.—Golgi apparatus (G) and dye droplets (B) in cells of convoluted tubules of the mouse kidney. These figures are arranged as a progressive series to illustrate what are regarded as successive stages in the deposition of the dye.

(Protocol of experiment—same as figs. 3 and 4.)

6. *Relation of Dye Droplets to Cytoplasmic Structures in Liver Cells.*

Essentially the same relationship has been found to exist between dye droplets and the cytoplasmic structures in parenchymatous cells of the liver, as in the cells of the tubules of the kidney. Fig. 11 shows vitally stained liver cells; fig. 12 the normal form of the Golgi apparatus (G) in such cells; and fig. 13 the intimate relationship between the dye (B) and the apparatus (G) when both are demonstrated in the same preparation. The absence of any clear relationship between the dye (B) and mitochondria (M) is illustrated in fig. 15.

It has been clearly established that during mitosis the Golgi apparatus fragments, and becomes scattered in the cytoplasm. There is a similar dispersal of trypan-blue droplets (B) in liver cells during mitosis, as is seen in fig. 14.

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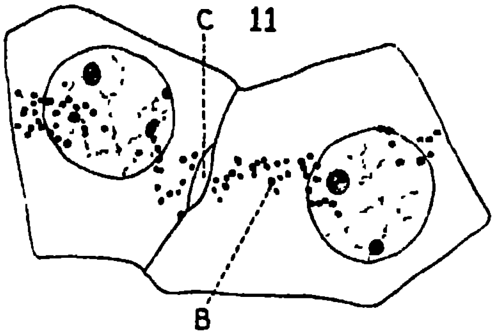

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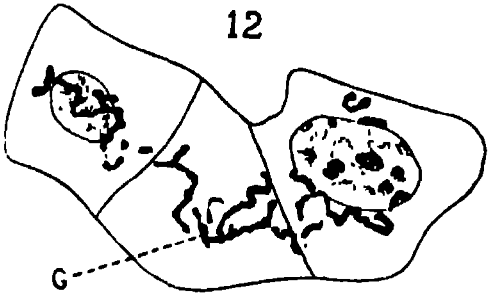

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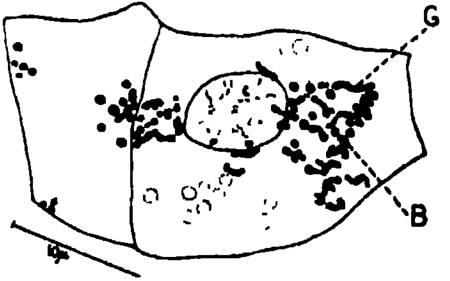

FIGS. 11–13.—Parenchymatous cells of the liver of the mouse.

FIG. 11.—Typical arrangement of dye droplets (B) after three injections of 0·5 c.c. of 1 per cent. trypan blue.

FIG. 12.—Peri-nuclear arrangement of the Golgi apparatus (G) in a normal mouse.

FIG. 13.—Golgi apparatus (G) and dye droplets (B), after three injections of 0·5 c.c. of 1 per cent. trypan blue.

To facilitate the study of the liver cells it has been found convenient to stain sections of modified Kopsch material with neutral red. The trypan-blue droplets are by this means counterstained red. Figs. 16 to 19 represent the result of the application of this technique. Figs. 16 and 17 are two cells cut at a plane at right angles to the longitudinal axes of two intercellular bile capillaries (C). The cells of fig. 18 are cut along the plane *x*—*y*

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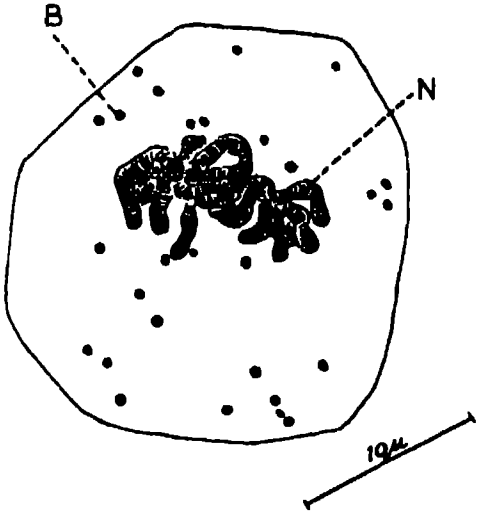

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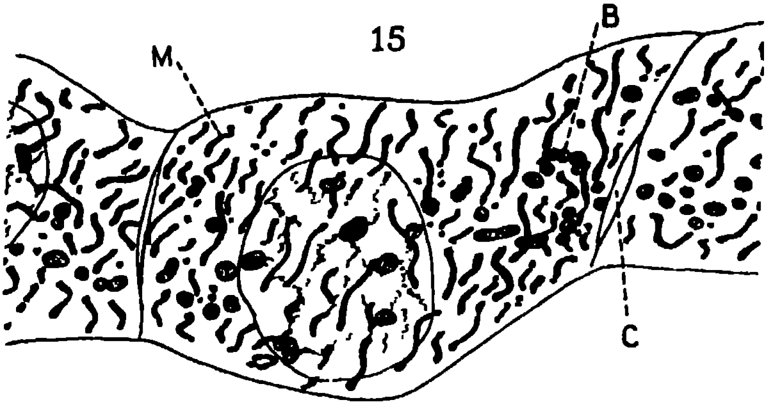


Figs. 14 and 15.—Parenchymatous cells of the liver of the mouse stained *intra vitam* with trypan blue.

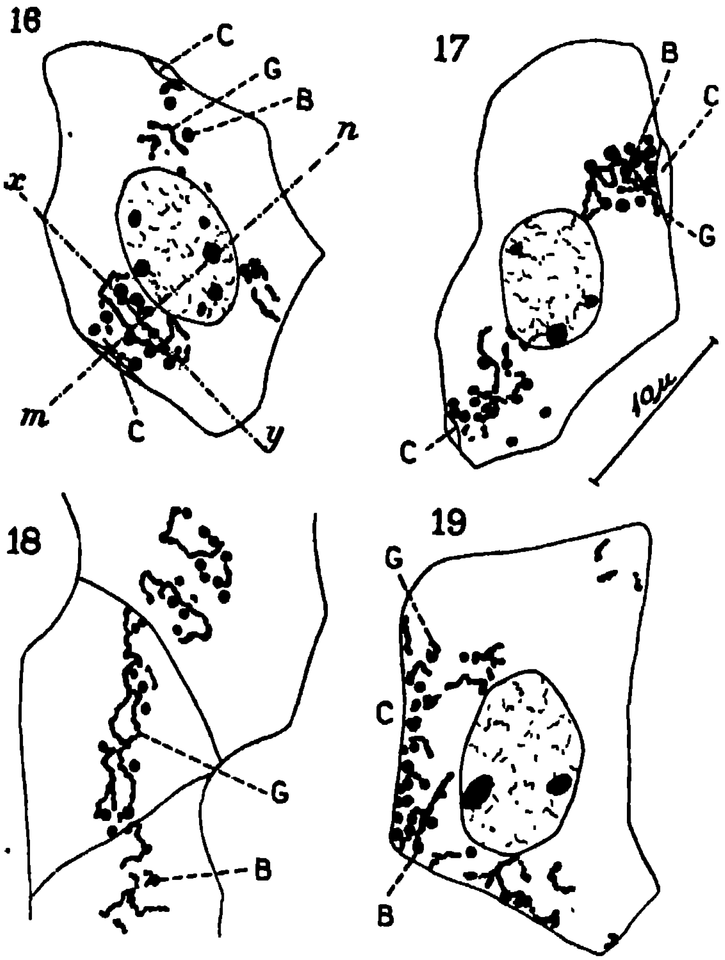
Fig. 14.—Metaphase of mitosis showing scattered dye droplets (B), and chromosomes (N).

(Protocol of experiment—1st day, injected subcutaneously 0·5 c.c. of ½ per cent. trypan blue. The same repeated on 3rd, 5th, 8th and 10th days. The tissues fixed on the 12th day.)

Fig. 15.—Mitochondria (M) and dye droplets (B).

(Protocol of experiment—Mouse received seven injections of 1 per cent. trypan blue over a period of 27 days.)

of fig. 16, and are looked at from the direction *m* or *n*; while the cell shown in fig. 19 has been sectioned along the plane *m*—*n* (fig. 16), and is looked at from the direction *x* or *y*. The Golgi apparatus (G) in all these cells, therefore, occupies a peri-nuclear position, and is directed



Figs. 16-19.—Golgi apparatus (G) and dye droplets (B) in parenchymatous cells of the liver of a mouse stained *intra vitam* with trypan blue. The dye droplets have been counterstained with neutral red in the sections. Figs. 16 and 17 are cells cut along a plane at right angles to two intercellular bile capillaries (C). Fig. 18 has been sectioned along the plane $x-y$ of fig. 16, and is looked at from the direction m or n. Fig. 19 has been cut along the plane $m-n$ of fig. 16, and is looked at from the direction x or y. (Protocol of experiment the same as for fig. 14.)

towards intercellular bile capillaries (C). The figures show that the dye accumulates as droplets (B) in relationship with the Golgi apparatus (G), preparatory to its excretion into the bile canaliculi.

7. *Function of Golgi Apparatus and Mitochondria.*

The result of our observations are entirely confirmatory of the view of Nassonov, that it is the function of the Golgi apparatus to bring about an elective concentration into droplets of the products of cellular activity. This process is a physical one. What, then, is the function of mitochrondria? This problem has been discussed recently by Horning and Petrie (1927). They refer to Cowdry's (1926) surface-film theory of the function of mitochondria, and express the opinion that in the light of recent biochemical work the evidence is strongly in favour of enzymatic syntheses taking place at the mitochondria-cytoplasmic surface. Their own studies upon mitochrondria during the germination of cereals support this conception. In their opinion a starch-splitting enzyme is located within, or at the surface of, the mitochondria of the cells of the scutellum of the maize grain. A remarkable process is described by these authors. During germination, the mitochondria of the scutellum cells increase in numbers, and are "secreted" into the starch-containing endosperm cells, where they bring about the "corrosion" of the starch grains by liberation of the enzyme.

There is a great deal of evidence to show that increased cellular activity is expressed morphologically by an increase in the cytoplasmic-mitochondrial surface, and also in that of the surface of the Golgi apparatus. An explanation of this is afforded by a correlation of the new conception of the function of the Golgi apparatus with the idea of the enzymatic function of the mitochondria. *At the mitochondrial-cytoplasmic surface synthesis by enzymes occur. The resulting products continually diffuse into the cytoplasm, preventing an accumulation at the surface of the mitochondria, which would inhibit further syntheses. At the surface of the Golgi apparatus the elaborated products are concentrated into droplets, as a preliminary to their elimination.* Such an hypothesis is in accordance with our present knowledge of these matters, and affords a crude explanation of the functional inter-relationship of the cytoplasmic organs.

8. *Summary.*

1. By means of the cytological technique described in this paper it is possible to demonstrate in cells of the kidney and liver of animals stained intra-vitally with trypan blue—

    (*a*) Dye droplets and mitochondria (figs. 3 and 15);
    (*b*) Dye droplets and Golgi apparatus (figs. 5–10 and 16–19).

2. No definite relationship can be established between dye droplets and mitochondria (figs. 3 and 15).

3. The dye droplets make their appearance in relationship with the Golgi apparatus, and when formed break away from it into the cytoplasm (figs. 5–10 and 16–19).

4. The formation of dye droplets in relationship with the Golgi apparatus resembles the process of formation of secretion granules in gland cells.

5. Regarded in the light of recent research these observations suggest that the following functional inter-relationship exists between the Golgi apparatus and mitochondria:—At the mitochondrial-cytoplasmic surface syntheses by enzymes occur. The resulting products continually diffuse into the cytoplasm, preventing an accumulation at the surface of the mitochondria, which would inhibit further syntheses. At the surface of the Golgi apparatus the elaborated products are concentrated into droplets preliminary to their elimination.

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A Theory of the Production of Zymase by the Living Cell.

By Egerton Charles Grey.

(Communicated by Prof. Sir F. Gowland Hopkins, F.R.S.—Received June 12, 1928.)

(From the William Dunn Institute of Biochemistry, Cambridge)

The writer puts forward the view that zymase is a modification of the respiratory mechanism, and that the type of fermentation which leads to the production of alcohol and carbonic acid, or alcohol and formic acid as ultimate products, is the result of the continued action, under anaerobic conditions, and in an aqueous medium, of this surviving group of respiratory enzymes. This view has, as far as the writer can ascertain, never been put forward in quite the same manner, although the trend of modern thought has undoubtedly been towards a conception of the close relation of respiration and fermentation.

The idea of Mazé (1904) seems to be the nearest approach to that here put forward, but differs in that like that of Stoklasa (1903) it supposes zymase to be ready formed in the cell, living aerobically, and alcohol to be formed normally in aerobic metabolism, and not as the result of anaerobiosis. The writer holds that the zymase does not exist as such, but is the result of a change in the structure of the respiratory enzymes. Mazé's view is in some degree opposed to Pasteur's theory that "*life without air*" is fermentation, and contrary to the observed facts that in many cases withholding oxygen is followed by a fermentation with the production of alcohol, while at a surface freely aerated, fermentation is greatly diminished. Pasteur (1872) found that mycoderma vini changed from the respiratory to the fermentative habit when plunged under water, and he observed, too, that the extent of alcohol production by yeast was inversely proportional to the aeration.

As early as 1821 Bérard had found that fruit maturing in an inert atmosphere gave rise to alcohol in very appreciable amount, which he attributed to "a sort of fermentation," and similar results were subsequently obtained by Lechartier and Bellamy (1869), Muntz (1878), Mazé (1899), and Godlewski and Polszeniusz (1901). Mazé, however, does not accept Pasteur's explanation of the absence of free oxygen as the exciting cause, for in his experiment upon peas, alcohol was produced even when air was in contact with the water in which the peas were submerged, and the separated cotyledons exposed to air gave a certain production of alcohol also. Moreover Mazé found that alcohol could be produced and oxidised by the same organism. Thus he found the ascomycete *Eurotyopis Gayoni* growing aerobically assimilated lactic acid, but submerged decomposed the acid into acetaldehyde and carbon dioxide.

The writer does not wish to emphasise the difference of his views from, but their similarity to, those of Mazé, and is chiefly concerned in bringing forward further evidence in support of the idea that zymase and the respiratory enzymes are very closely related. For if it is so, many interesting conclusions may be drawn which follow logically from the theory.

The evidence which the writer has to bring forward is the outcome of a long series of studies upon bacterial fermentation, chiefly by *Bacillus coli communis*. The similarity of the process of alcohol production by this organism with alcohol production by yeast, or by maturing fruit, or other parts of living plants, was pointed out by the writer (1913) as the consequence of the detection of acetaldehyde, which was shown to be produced only by the organisms which had been grown normally, and not by such as had been selected in the presence of chloroacetates, a treatment which Penfold (1911) had found robbed bacteria of the power to produce gas. Bacteria differ from yeast in their manner of decomposing sugar in particular in that they produce free hydrogen with the carbon dioxide. According to the third equation of Neuberg (1920), yeast converts alcohol into essentially the same products as does *B. coli communis*, with the exception that glycerine appears instead of lactic acid and hydrogen.

The writer has, however, shown in 1917, and in the later numbers of this series, that lactic acid production by *B. coli communis* is independent of the formation of the other products, which are formed by a separate enzyme mechanism. This mechanism, therefore, differs only from zymase in that it has not the capacity to reduce acetaldehyde so completely to alcohol; the hydrogen responsible for this reduction appears instead in combination with carbon dioxide as formic acid. The change in the case of yeast is

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$

and for the bacterium,

$$H_2O + C_6H_{12}O_6 = C_2H_6O + C_2H_4O_2 + 2CO_2 + 2H_2$$

and for yeast in an alkaline medium,

$$H_2O + 2C_6H_{12}O_6 = C_2H_6O + C_2H_4O_2 + 2CO_2 + 2C_3H_8O_3.$$

It is clear that the resemblance between these types of fermentation is so close that one is justified in making generalisations concerning zymase from the study of yeast or bacteria.

Experimental.

The writer has shown in the previous communication, which was a confirmation of the experiment described in Part III (1917) of this series, that when an emulsion of *B. coli communis* (aerobically grown) is added to glucose, there follows immediately under anaerobic conditions a decomposition of the glucose into a group of products among which alcohol is conspicuous, but that under these circumstances practically no lactic acid is formed. During this period the organisms are apparently dying in large numbers. They have at any rate lost the power to form colonies on nutrient agar. These organisms have clearly been adapted to an aerobic existence, and when plunged into the liquid medium, in absence of air, they produce what is virtually an alcoholic fermentation. In the next few hours, there follows an apparent or real increase in the number of living or viable organisms; there is a synthesis of carbohydrate, and this is followed by lactic acid production. We may explain the formation of lactic acid later in the fermentation by saying that the organisms have taken on this mode of fermentation in response to the environment, or that unable to bring about effectively the formation of alcohol they fall back, so to speak, on the simpler mode of lactic acid production; but in any case, we reach the same conclusion, namely, that when recently grown in air the organisms have a power to produce alcohol which they do not possess after they have been out of contact from the air for some time, although they still ferment sugar at approximately the same rate.

The bacteria under these conditions, therefore, seem to the writer to have behaved much as the mycoderm studied by Pasteur, or the fruit placed by Bérard in an atmosphere of carbon dioxide, or the peas of Mazé placed under water. On account of the two distinct modes of anaerobic fermentation possible to *B. coli communis*, this organism is particularly suitable for demonstrating the connection which exists between alcoholic fermentation, and aeration.

Two further experiments may now be described in which again an analysis of the products of decomposition of glucose has been made at short intervals. The analysis of the products of the fermentation is shown below. To arrive at the results given in the table it was necessary to deduct from the amount of each substance present at the end of any period, the amount of it which had been present at the beginning of the period. The figures given in the table thus refer to the amounts of the various products which have appeared during the period in question.

Table I.—Products of the Decomposition of Glucose by *B. coli communis*, during Different Periods of the Fermentation.

	0–6 hours.	6–25 hours.	25–38 hours.	38–58 hours.	58–83 hours.
Hydrogen	None	0·195	0·048	0·263	0·137
Carbon dioxide	4·715	None	None	5·740	2·795
Formic acid	1·380	1·214	1·095	0·175	—0·455
Acetic acid	7·920	7·092	4·446	8·220	5·016
Succinic acid	None	2·236	2·620	2·095	—0·183
Alcohol	1·350	2·067	1·220	0·934	0·344
Lactic acid	1·340	6·678	2·736	6·696	8·964
	16·705	19·495	12·168	24·139	17·365
Glucose consumed	18·3	37·1	4·8	28·0	11·0
Total glucose consumed					99·2
Total products recovered					89·9

As in the previous experiments of this type described by the writer (1917 and 1928), the weight of the products obtained in the first period is approximately the same as the weight of the sugar fermented in this period. When working with an emulsion of bacteria grown on agar aerobically, the writer has found no exception to this rule. There is a rapid death of bacteria in this period, but none the less the rate of fermentation is approximately the same as in later periods. The writer interprets these phenomena to mean that the fermentation during the first period is carried out through the agency of enzymes present in the bacteria at the time they are taken from the agar, in other words at the time when the bacteria are living an aerobic existence. By comparing, therefore, the products of Period I with those of later periods we should be able to judge which are formed by enzymes generated during aerobic existence and which by enzymes generated or made to function later, *i.e.*, during anaerobic existence.

In the second period it will be observed 37·1 grams of glucose have been consumed, though the products in this period amount only to 19·5 grams. The missing glucose appears, however, in the products of the third period. The explanation has already been given in connection with previous experiments of this type, with which this present experiment is in entire agreement. The glucose which has disappeared has been converted into a non-reducing polysaccharide, stored up in the bacterial cells. In every experiment which the writer has carried out, and also in experiments carried out with W. M. Colles (1924), this polysaccharide has been shown to be formed in considerable amount during the second phase of the experiment, namely, when the organisms having adapted themselves to the medium are increasing in number. This period is either accompanied by or immediately followed by an abundant formation of lactic acid.

We are, therefore, apparently witnessing two kinds of fermentation occurring to some extent simultaneously, the one a direct fermentation of glucose into formic acid (or its gaseous products), acetic acid, succinic acid and alcohol, and the other a fermentation of polysaccharide into lactic acid. It is not, however, certain that the lactic acid is only formed subsequently to the polysaccharide synthesis, it may also be formed possibly directly from glucose, and likewise the polysaccharide may yield other products than lactic acid.

The relative proportions in which the products are formed appear more clearly in Table II, in which they have been calculated in percentages upon the totals of products in each period.

Table II.—Products of the Decomposition of Glucose by *B. coli communis*, during Different Periods of the Fermentation, calculated as Percentages upon the Total Products of the Periods.

	0–6 hours.	6–25 hours.	25–38 hours.	38–58 hours.	58–83 hours.
Hydrogen	None	1·00	0·39	1·09	0·79
Carbon dioxide	28·22	None	None	23·78	16·10
Formic acid	8·26	6·23	9·00	0·73	—
Acetic acid	47·41	46·38	36·54	34·06	28·89
Succinic acid	None	11·47	21·53	8·68	—
Alcohol	8·08	10·60	10·08	3·87	1·98
Lactic acid	8·02	34·26	22·48	27·74	51·62

In the first period, it will be observed, there has been a formation of approximately 8 per cent. lactic acid. It is true that the alcohol formed in this period also only amounts to 8 per cent., but we must consider the fact that acetic acid is an alternative for alcohol, for both may be derived from acetaldehyde. The essential fact, therefore, is that in this first period the products of acetaldehyde have been formed, in amount representing 55 per cent. of the sugar decomposed, and since we know that formic acid or carbon dioxide and hydrogen must arise in conjunction with the formation of acetic acid it follows that about 92 per cent. of the glucose has in the first period been transformed by a reaction which may be represented by the equation

$$C_6H_{12}O_6 = C_2H_4O + 2CO_2H_2.$$

The above change demands the formation of about 44 per cent. CO_2, either free or as formic acid, whereas about 36 per cent. has actually been obtained. Part of the acetic acid may have been derived by oxidation of acetaldehyde, for it is clear that oxidation is occurring during this period, while at the same time a very unstable compound of high reducing value is formed. The disappearance of hydrogen in this first period indicates also the intramolecular oxidation which is occurring. It would complicate matters to discuss these details further here.

It is also possible that part of the acetic acid may arise by a reaction independent of acetaldehyde formation, but the simplest view is that which regards alcohol, acetic acid, and probably also succinic acid when it appears, as derived by the same set of reactions, with acetaldehyde as the mother substance formed in conjunction with formic acid, or carbon dioxide and hydrogen. The above reaction will be seen to be essentially an alcoholic fermentation using the expression in the broad sense as including the various alternative reactions based upon acetaldehyde.

In the fifth period, on the other hand, the carbon dioxide formed amounts to less than 14 per cent. and the acetic acid plus alcohol to 30 per cent. or a total of 44 per cent. of products, which may be formed in conjunction with acetaldehyde, while 56 per cent. of the sugar has become lactic acid.

It is conceivable, as mentioned above, that acetic acid may in part arise independently of formic acid by a mere intramolecular change of glucose, since acetic acid has the same empirical formula as glucose. The arguments which have been brought forward, however, would not be weakened by admitting that some of the acetic acid, namely, that part which is formed in excess of the carbonic acid or formic acid produced, had a separate origin from glucose. It would suffice to consider the gradual diminution in the production of alcohol and the increase in the production of lactic acid to become convinced of the fact that alcoholic fermentation by *B. coli communis* is more readily brought about by organisms which have been recently derived from aerobic conditions, than by those which have been some time in contact with the medium under anaerobic conditions.

By way of further confirmation of the results embodied in Tables I and II, another experiment of the same kind may be described. The percentage results alone need be given (Table III). In this experiment 32·32 grams of glucose were fermented and 32·03 grams of products obtained.

Table III.—The Products of the Decomposition of Glucose at Successive Periods throughout the Fermentation by *B. coli communis*, expressed as Percentages upon the Total Products of the Period.

	6 hours.	12 hours.	24 hours.	36 hours.	48 hours.
Hydrogen	None	None	1·30	0·53	0·18
Carbon dioxide	28·2	None	29·5	35·2	22·9
Formic acid	15·5	None	3·3	None	None
Acetic acid	20·4	35·5	4·3	25·7	32·4
Succinic acid	25·7	46·9 ?	33·2	None	7·0
Alcohol	6·9	11·5	17·0	None	4·8
Lactic acid	3·3	6·1	11·3	38·5	32·7

The results of this experiment are essentially in agreement with those of the previous experiment. Here the alcohol production rises to a maximum in about 24 hours, during which time the lactic acid production is dormant. The sudden disappearance of alcohol appears to be due in part to its conversion into acetic acid, which is observed here, as in the previous experiment, in spite of the anaerobic conditions.

The formation of carbon dioxide without hydrogen deserves special note, for it is a fact observed in both experiments. It is the extreme case of the phenomenon observed in most of the writer's experiments, namely, that carbon dioxide is formed in excess of hydrogen. It would appear to signify that carbon dioxide does not necessarily arise from preformed formic acid, but possibly there is from the very outset an equilibrium which may be represented thus

$$H_2 . CO_2 \rightleftarrows H\,COOH.$$

This raises a most interesting question as to the origin of carbon dioxide in these fermentations, which, however, cannot be gone into here. Other evidence of a similar kind will be found in Part IV (1920) of this series. The fact, however, that alcohol is formed by bacteria in conjunction with carbon dioxide, instead of formic acid, and without necessarily an evolution of hydrogen, illustrates still further the closeness of the parallel between alcoholic fermentation by bacteria and by yeast.

Too much stress must not be placed upon the figures for succinic acid. The writer has previously found pyruvic acid and also an unstable acid formed at the beginning of the fermentation which is not yet characterised, but might in part appear in the succinic acid fraction. Details will be communicated later.

The doubt might still be entertained as to whether the gradual diminution in alcohol production and increase in lactic acid production were the result of an actual change in the bacterial enzymes, or were due to the inhibiting action of the products accumulating in the medium. Against this idea, however, are the facts, confirmed by several experiments, that after an initial period in which there is death of bacteria, there follows a period of growth and synthesis, evidenced by the number of colonies which grow on agar, and also by the formation of the polysaccharide.

These facts suggest that the initial enzyme equipment of the air-grown bacteria is not suitable for prolonged anaerobic existence and that a readjustment occurs, presumably with the formation of new enzymes. The criticism, however, would be just, and must be answered by further facts.

In anticipation of the criticism, the experiment, described here as No. 1, was so arranged that during the course of the main fermentation, already described (Tables I and II), samples of the fluid could be removed under anaerobic conditions into flasks containing fresh glucose, chalk and the necessary salts used for the fermentation. These constituted side fermentations, which should all give approximately the same products if the enzymes were the same at the time of removing the samples, but which should give different results if the enzymes were different. The times at which the samples were removed to begin the side fermentations were 24, 38 and 58 hours respectively.

Table IV.—Side Fermentations set up in Fresh Glucose by means of Liquid drawn off from the Main Fermentation (Table I). (Results expressed as Percentages upon the Total Products.)

	A.	B.	C.
Hydrogen	0·96	1·26	1·16
Carbon dioxide	19·44	16·91	13·68
Formic acid	4·00	0·43	0·18
Acetic acid	28 48	26·67	20·76
Succinic acid	4·23	4·56	0 75
Alcohol	4·60	2·94	1·50
Lactic acid	38·28	47·22	61·95

The fact, therefore, that the side fermentations do not all show the same results, although the conditions were the same in all three, indicates that each has continued the fermentation in the way it was being carried out in the main experiment at the time the sample was removed. At the time A was begun, the main fermentation had been in progress 24 hours. Alcohol production was therefore already on the wane and lactic acid production on the increase. Accordingly, in the side fermentation A, we find a proportion of lactic, not as at the beginning of the main fermentation but much higher, the proportion rising progressively in the side fermentations B and C, since these were removed at still later stages of the main fermentation. Conversely, as was to be anticipated, the production of carbon dioxide and formic acid and alcohol have gradually decreased in passing from A to C. Thus the alcohol in A is nearly twice that of B, and B twice that of C.

Another point of interest which emerges from the study of these side fermentations is that the rate of fermentation is progressively slower, the later the samples used to start them were removed from the main fermentation. That the main fermentation should slow down might be attributed to the inhibitory effect of end products upon the enzymes, considered to be otherwise intact, but that the side fermentations set up by samples taken from the main fermentation should become progressively slower, can only be interpreted to mean that a change is taking place in the proportion between the enzymes themselves during the progress of the fermentation.

Conclusion.

1. Of the two modes of fermentation by which *B. coli communis* brings about the degradation of carbohydrates, namely, lactic fermentation, and the modified alcoholic fermentation, the latter is possible only when the organism has been recently grown in the presence of free oxygen. This does not mean that oxygen can be dispensed with in the production of the lactic acid enzyme, but that this mechanism can survive longer in the absence of oxygen than can the alcohol forming mechanism, which is considered in the case of bacteria to be very similar if not identical with yeast zymase.

2. As an outcome of the observations on alcohol production by bacteria, the generalisation is made that "zymase is the surviving portion of the respiratory mechanism" and alcoholic fermentation is "the continued action, under anaerobic conditions, and in contact with an aqueous medium, of the surviving portion of the respiratory mechanism."

3. Thus zymase is not considered to be ready formed in cells growing aerobically, but, on the contrary, its formation is incompatible with respiration since it is supposed to arise by a distortion of the respiratory mechanism, and respiration will therefore fall off in proportion as zymase is formed. It is useless therefore to look for zymase in cells living a normal aerobic life. It is particularly in respect of this last paragraph that the writer's view of the nature and origin of zymase differs from that put forward by any other.

In conclusion, the writer wishes to express his best thanks to Prof. Arthur Harden, F.R.S., for his very helpful criticism, and to Sir Frederick Gowland Hopkins, in whose laboratory this work was completed, for his constant help and encouragement.

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The Enzymes of Bacillus Coli Communis. *Part* VI.—*The Alternative Modes by which* B. Coli Communis *may bring about the Anaerobic Decomposition of Glucose.*

By Egerton Charles Grey.

(Communicated by Sir Frederick Hopkins, F.R.S.—Received June 12, 1928.)

(From the William Dunn Institute of Biochemistry, Cambridge.)

The previous work which has been described in this series, has led to the recognition of the independence of many of the enzymes of *Bacillus coli communis* (see this series, 1914, 1917, 1920) and finally to a simple theory of bacterial fermentation in general (see this series, 1924). While admitting that some of the enzymes of bacteria may be specifically adapted to act upon certain configurations of atoms, this theory postulates that there exist within the bacteria cells, in all cases, enzymic mechanisms of a more fundamental character, a few of which suffice to bring about a great variety of chemical changes. These fundamental mechanisms divide themselves into two groups —those connected with intramolecular oxidations and reduction, and those concerned with the separation of carbon atoms. Stabilisation of the products of primary cleavage is considered to occur automatically and not to require additional enzymes. The course of any fermentation would depend upon the relative rates of action of these two fundamental modes of decomposition. The rate of action of the oxidation-reduction mechanism would moreover be conditioned by the availability of acceptors for oxygen and hydrogen, and hence this mode of decomposition is more complex and not so independent of the conditions as the latter mode, which brings about simple cleavage and intramolecular rearrangement of atoms.

If this theory is correct it should follow that when the conditions of life for a micro-organism are difficult (as for example when the nutrition is poor, when there is prolonged anaerobiosis, and when the organism is old, or enfeebled by toxic influences) it should bring about less oxidation and reduction, and more simple cleavage and intramolecular rearrangement of the substrate. In the case of *B. coli communis*, therefore, less alcohol, formic acid, succinic acid and carbon dioxide, and more lactic acid. The case of acetic acid cannot be considered for the moment, as it might conceivably arise by either mechanism.

A consideration of the facts described in the previous communications of this series, will probably bring conviction that they accord well with the theory; for example, in Part III (1917), it was shown that when a fresh aerobic growth of bacteria was allowed to ferment glucose anaerobically, abundant alcohol, succinic acid, formic acid, and carbon dioxide were formed, but practically no lactic acid. When, however, the same organisms had been acting upon the sugar anaerobically for about 24 hours they entered upon a phase in which they converted the glucose chiefly into lactic acid. The result seemed to the writer of such importance that he decided to repeat the whole experiment described in Part III (1917).

Experimental.

The experiment was begun on February, 1928. The organism used was the stock *B. coli communis* obtained from the National Collection of Type Cultures, the Lister Institute, London. For the purpose of the experiment an emulsion of the organism was obtained from an aerobic growth on nutrient agar disposed in a broad layer in Roux bottles. The solution used to wash the bacterial growth from the agar consisted of tap water to which 6 grams of potassium sulphate and 1 gram of magnesium sulphate were added per litre. The reasons for these additions are given in Part III of this series (1917).

Into a flask of 8840 c.c. capacity were introduced 7000 c.c. of tap water, 42 grams of potassium sulphate, 7 grams magnesium sulphate and 100 grams of glucose. 100 grams of glass wool was also used to serve as a support for the chalk added later. The solution was sterilised by heat, and after cooling to about 40° C., 100 grams of sterile chalk was added, and finally 1000 c.c. of the emulsion of bacteria. The flask was connected to a gas-collecting apparatus of the type described in Part III of this series, and the air above the liquid displaced by a current of nitrogen, after which the fermentation was allowed to proceed at 37° C. and samples were removed from time to time during the course of the fermentation for the purpose of analysis.

The experiment was begun at 5.30 p.m. on February 28, 1928. Bubbles of gas were first observed at 7 p.m. while a brisk fermentation had set in by 11.30 p.m. Times of removing samples for analysis, volumes of the samples, and counts of numbers of organisms apparently living per cubic centimetre at time of taking the samples, may be seen from the following figures.

Data for Experiment begun 5.30 p.m. on February 28, 1928.

No.	Time of taking sample.	Time since beginning of experiment.		Volume of sample.	Volume of solution remaining.	Number of organisms apparently living per cubic centimetre.
		h.	m.	c.c.	c.c.	
1	11.30 p.m. on 28th	6	0	788	7212	1×10^{10}
2	6 0 a.m. on 29th ..	12	30	982	6230	7×10^{7}
3	5.0 p.m. on 29th .	23	30	1970	4260	1×10^{7}
4	1.0 a.m. on 30th	31	30	965	3295	$<1 \times 10^{6}$
5	7.0 a.m. on 30th	37	30			

The rapid death of bacteria which accompanies the fermentation under these conditions is worthy of special mention, for when the organisms are suspended in saline, without any added nitrogenous matter, the bacterial count does not alter markedly in 24 hours. The act of fermentation appears to be responsible for the death of the micro-organisms, and this phenomenon in itself is in the writer's opinion of considerable physiological significance.

The estimation of the total carbon dioxide produced in each period of the fermentation by the decomposition of glucose is somewhat involved, it necessitates the estimation of the total carbon dioxide produced, and the subtraction from this of the carbon dioxide resulting through the action of acids on the chalk, and also in cases where there is a decomposition of formic acid previously formed, a further deduction on this account must be made.

To ascertain the total amount of carbon dioxide produced in each period of the experiment we must add together the amount of gas evolved and collected in the sodium hydroxide, the amount remaining in the gas space, which in the present experiment is gradually increasing, and the amount dissolved in the liquid, and from this total must be deducted the amount of gas left over in the liquid and in the gas space from the previous period of the experiment.

The following are the data for the evolution of carbon dioxide gas in this experiment :—

The Production of Carbon Dioxide at Different Intervals of the Fermentation.

(1) Carbon Dioxide in the Gas Space in the Flask.

Period.	Volume of gas space.	CO_2.	Volume CO_2 present.	CO_2 formed during period.	Corrected volume.	Equivalent in cubic centimetres NCO_2.
	c.c.	Per cent.	c.c.	c.c.	c.c.	c.c.
1	840	20	168	168	150	13·6
2	1628	42	684	516	459	41·7
3	2610	33	870	186	166	15·1
4	4580	24	1099	229	204	18·4
5	5545	33	1848	749	666	60 5

(2) Carbon Dioxide in the Fermentation Liquid.

Quantities in cubic centimetres of normal carbonic acid.

Period.	Volume of the liquid.	Amount per litre.	Total cubic centimetres.	Brought forward.	Produced in the period.
	c.c.	c.c.	c.c.	c.c.	c.c.
1	8000	17 6	140·8	None	140·8
2	7212	33·3	240·4	126 7	113·7
3	6230	40 0	249 2	207·7	41·5
4	4260	40 0	170 4	170·4	None
5	3295	43 6	130·5	131·8	None

Calculation of the Total Carbon Dioxide Produced from Glucose during each Period.

Period.	Collected in alkali.	Remaining in gas space.	Remaining in fermentation liquid.	Total formed.	Formed from chalk.	Formed from glucose.
1	2·5	13 6	140 8	156·9	163	None
2	10·3	41·7	113·7	165·7	−27·4	193·1
3	26 5	15 1	41 5	83 1	84·1	None
4	29·2	18 4	None	47·6	40·9	6 7
5	55·9	60·5	−1·3	115·1	60·3	54·8

The fermentation therefore in the first period produces no carbon dioxide. In the second period there is an abundant production of carbon dioxide. In the two subsequent periods the carbon dioxide production falls to practically nothing, but rises again in the last period.

There is an anomaly in the absence of carbon dioxide in period three, because hydrogen is produced in this period. There can, however, be no question, taking the figures as a whole, that the rate of production of carbon dioxide during the fermentation varies from period to period. As will be seen from the analysis of the other products the absence of carbon dioxide in the first period is accounted for by an accumulation of formic acid, there is therefore nothing anomalou in the absence of this gas at the outset of the experiment.

The calculation involved in the estimation of the amount of hydrogen produced in each period is somewhat similar to the calculation of the carbon dioxide, but simpler. The details need not be given here, but only the final result.

Production of Hydrogen Gas from Glucose during each Period of the Experiment compared with Carbon Dioxide Gas Production.

	1.	2.	3.	4.	5.
		c.c.	c.c.	c.c.	c.c.
H_2	None	Trace	638	803	1102
CO_2	None	2143	None	74	605
Time	6 h. 0 m.	23 h. 30 m.	23 h. 30 m.	31 h. 30 m.	37 h. 30 m.

Total hydrogen	2543 c.c. gas.
Total carbon dioxide	2822 c.c. gas.

The experiment therefore shows that hydrogen does not appear till later in the fermentation than the carbon dioxide. It appears to be held up at first and liberated gradually as the fermentation proceeds.

The appearance of carbon dioxide at the beginning of the fermentation unaccompanied by hydrogen has been observed by the writer in several experiments carried out at different times during the last few years. There is no question but that this holding up of the hydrogen is due in part to the formation of a highly unstable compound, which has in consequence a high reducing value. In consequence the estimation of sugar in the solution at the early stages of the experiment by the copper reduction method gives rise to very high results, as also does the estimation of formic acid by a reduction method. The sugar must be estimated after prolonged heating with dilute acid, under which circumstance the unstable compound is decomposed. The nature of this substance is under investigation.

In spite of the varied rates at which carbon dioxide and hydrogen appear during the course of the experiment, the total volume in either case is approximately the same, carbon dioxide being slightly in excess. The ultimate proportion in which these gases appear in the experiment is the same as in the experiment of Harden (1901).

The complete data relating to the products of decomposition of glucose in this experiment are given in Table I.

Table I.—Products of Decomposition of Glucose formed during Successive Periods of a Fermentation by *B. coli communis.*

Time.	0–6 hours.	6–12 hours.	12–23 hours.	23–31 hours.	31–37 hours.
Hydrogen .	None	Trace	584 c.c. 0·0523	730 c.c. 0·0654	991 c.c. 0·0888
Carbon dioxide	None	4·248	None	0·147	1·206
Formic acid	4·304	−1·329	0·833	0·313	0·409
Acetic acid	None	None	1·344	0·486	0·888
Succinic acid	1·634	−0·738	0·926	0·116	0·630
Alcohol	1·069	0·290	1·204	0·759	0·431
Lactic acid	0·108	2·178	2·092	2·143	2·061
Totals	7·105	6·716	6·399	3·964	5·625
Sugar decomposed	7·04	12·40	0·37	7·58	3·49

Total sugar fermented .	30·88 gr.
Total products obtained	20·81 gr.

There can be little doubt that the analysis as a whole is correct. The products add up nearly to the total of sugar fermented, and as regards the main facts the results of this experiment confirm well the experiment described by the writer in Part III of the series (1917).

It may be seen from the data given above, as from those given in Part III of this series (1917) that the first phase of the fermentation is concerned with the production of alcohol, formic acid, and succinic acid; and as before, it will be noticed that there is no synthesis during this period, the weight of sugar fermented being recovered in the products formed during the same period. In the second period three phenomena are observable, sugar is apparently decomposed without yielding products in equivalent amount during the same period; the sugar has in reality been transformed into a non-reducing material which is fermented during the subsequent period, as may be seen from the figures. Secondly, there is a decomposition of products formed in the first period, namely, formic acid, and succinic; and thirdly, lactic acid is being formed, and is subsequently formed continuously till the end of the experiment.

The extent to which reducing sugar has been converted into non-reducing polysaccharide, may be deduced from the following figures for the glucose estimation in 1000 c.c. of the fermentation fluid, before and after hydrolysis, at different periods throughout the experiment.

Time from start.	Glucose value.	
	Before hydrolysis.	After hydrolysis.
h. m.	(per 1000 c.c.)	(per 1000 c.c.)
6 0	12·03	11·62
12 30	9·94	9·90
23 30	7·85	9·84
31 30	7·00	8·06
37 30	7·05	6·60

The presence of non-reducing polysaccharide is masked in the second period by the presence of a highly reducing substance, and this is why in the first period also there appears to be more sugar before than after hydrolysis. This highly reducing material is destroyed in the second and third periods, but appears in small quantity in the last period. It is interesting to note that the fermentation of glucose stopped at the end of the third period, although there was still 7 grams of sugar per litre. This result confirms the finding that the liquid contained very few living bacteria. Subsequently to the third period the products of fermentation are chiefly due to the decomposition of the polysaccharide previously synthesised. We have here apparently a fermentation analogous to the auto-fermentation of yeast, in which glycogen is being utilised.

The writer would like here to record an observation made by his colleague Mr. W. M. Colles (1924), namely, that the process of autolysis of the polysaccharide of *B. coli communis* is not inhibited by toluene. This is a further point of analogy with yeast (see Harden 'Alcoholic Fermentation,' p. 33).

To bring out more clearly the nature of the chemical changes occurring at the various stages of fermentation, we may write the data given in Table I in the form of percentages upon the total of products formed in each period. This gives us Table II.

Table II.—The Products of Decomposition of Glucose at Successive Periods of Fermentation, expressed as percentages of Total Products formed in the same period.

	0–6 hours.	6–12 hours.	12–23 hours.	23–31 hours.	31–37 hours.
Hydrogen .	None	None	0·8	1·6	1·6
Carbon dioxide	None	63·3	—	3·6	21·1
Formic acid	60·6	None	12·9	7·8	7·2
Acetic acid	None	None	20·8	12·1	15·5
Succinic acid	22·8	None	14·4	22·9	11·0
Alcohol	15·1	4·3	18·6	18·8	7·6
Lactic acid	1·5	32·4	32·5	53 2	38·0

It may be pointed out that the alternative modes of glucose decomposition here described are more fundamental, so far as the existence of the bacterium is concerned, than the alternative modes of fermentation which Neuberg has described for yeast. (See Neuberg and Reinfurth, 1918, Neuberg and Hirsch, 1919, Neuberg, Hirsch and Reinfurth, 1920.) The second and third modes of fermentation set up by yeast are essentially modifications of the zymase action; they possibly occur to a slight extent with normal yeast fermentation, for glycerol and acetic acid are constant products of yeast fermentation, but little of these products is formed. The reactions do not appear to be indispensable to the life of the yeast, as is lactic acid production to the life of a bacterium of the Coli type. The readiness with which the enzyme mechanism responsible for gas production in the Coli group of bacteria is inhibited, illustrates the ability of the bacterium to dispense with this mode of fermentation.

Penfold (1911) showed that growth of coliform bacteria on agar, containing about 1 per cent. of sodium monochloroacetate, gave rise to colonies many of which had lost the power of gas formation. As a result of the change the organisms, as Harden and Penfold (1912) showed, produced lactic acid in the place of alcohol and other products associated with gas production. That the change, however, had not fundamentally modified the organism was indicated by the fact that only with difficulty could the organism be prevented from reverting to its former fermentative habit of gas production. The writer (1914) examining the same organisms concluded that the effect of the chloroacetate treatment had been to inhibit, or temporarily abolish, the reducing mechanism, in other words to lower the *r*H (Mansfield Clerk) of the cell.

Conclusions.

The claim made in Part III (1917) of this series that fermentation by *Bacillus coli communis* could be brought about in more than one way, though under anaerobic conditions, and that the mode of fermentation depended upon the vitality of the organisms, has been confirmed by a further study of the products of fermentation at short intervals.

Two main modes of anaerobic fermentation by *B. coli communis* are distinguishable, the one involving cleavage combined with oxidation and reduction, and the other involving cleavage combined with mere molecular stabilisation. The former is analogous to alcoholic fermentation by yeast, with the chief fundamental difference that formic acid appears instead of carbonic acid. The latter is essentially a lactic acid fermentation. *B. coli communis* thus appears to combine the enzymic properties of yeast with those of a lactic acid bacillus.

The latter mode of decomposition is the simpler; it supplies less energy to the cell, but also makes less demands upon the resources of the cell, and appears to be less sensitive to the influence of the reaction of the medium; consequently it is the mode of fermentation adopted by the organism when its vitality is low, as the result of age, prolonged absence of oxygen, and accumulation of toxic products. The action of chloroacetates observed by Penfold and Harden (1912) finds its explanation on this basis.

My best thanks are due to Prof. Arthur Harden for valuable criticism, and to Sir Frederick Hopkins, F.R.S., in whose laboratory this work was done, for valuable help and advice.

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On Inhibition as a Reflex Accompaniment of the Tendon Jerk and of other Forms of Active Muscular Response.

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(From the Physiological Laboratory, Oxford.)

[Plates 7–8.]

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Introductory.

In the condition of decerebrate rigidity the extensor muscles involved are in a state of tonic contraction, which is accompanied by a series of small action-currents, first demonstrated by Dusser de Barenne (9) and Buytendijk (4). Fulton and Pi-Suñer (12) have recently observed that these action-currents cease during the course of a tendon jerk in the same muscle, and that their reappearance precedes by a constant interval, and therefore is almost certainly the action-current accompaniment of the small mechanical contraction which is usually seen delaying the decline of the decerebrate tendon jerk. This mechanical contraction on the decline of the tendon jerk was known to earlier workers (24) as the tonic after-discharge of the jerk, and was described as the "hump" or "myotatic appendage" of the jerk, by Ballif, Fulton and Liddell (2). It was interpreted by them as a reflex contraction caused by the sudden relaxation of the jerk, a phenomenon of stretch-reflex type caused by the passive lengthening of the muscle during the relaxation.

Fulton and Pi-Suñer (12) interpret the absence of action-currents during the jerk as the result of slackening of some receptor organ in the muscle by the mechanical shortening taking place during the tendon jerk, and their reappearance as due to the reflex result of renewal of tension upon that receptor. This receptor, which, *ex hypothesi*, must react to passive stretch by causing reflex excitation in the same muscle, these latter authors identified with the muscle spindle, since in their opinion that organ, in virtue of lying "in parallel" with the muscle fibres, was likely to be slackened when the muscle substance surrounding it contracted.

This period of absence of action-currents during the tendon jerk has also been investigated in the course of experiments made by the author in work in connection with the problem of reflex posture, and since a number of facts obtained throw open another interpretation of the phenomenon they are here presented.

Technique.

The animal preparation, decerebrated under deep chloroform-ether anæsthesia, or in some experiments simply decapitated under the same anæsthetic, has the muscle or muscles which are to record isolated, by paralysis of all others by nerve section, except for the M. Ileopsoas and the short hip extensors, which are immobilised by tendon section. The origin of the muscle producing the record is fixed by drills through the bone, two for each bone in the case of the femur and tibia, and a clamp to the symphysis pubis where the pelvis requires fixation, and these drills and clamps are as usual fastened to steel uprights from the heavy experimental table so that complete rigidity of origin is obtained. The muscle tendon, with a piece of natural bony insertion, is hooked to the horizontal myograph lever and pulls downward upon it. Torsion wire myographs, as described by Sherrington (25), fixed rigidly to a strained steel girder framework, as devised by him and used in this laboratory for several years, have been used throughout. The myograph has been used in the same optical system as a string galvanometer of the Cambridge pattern. The present arrangement, which has worked satisfactorily for over eighteen months, is a double myograph, composed of two parallel wires with horizontal tension arms and parallel vertical registering frames. Each frame is of spring brass, flat in the plane of movement, and shaped to carry a taut fine rabbit hair which, magnified 100 times, forms the recording shadow. The vibration frequency for a free release from maximum tension is over 1000 d.v. a second. Damping when free is very rapid, owing to the friction bearing at the free end of the wire, and when attached to a viscous muscle mass appears to be instantaneous.

Combined with the two myograph images in the one optical system are the images of two strings in the double string case of the string galvanometer, and by arranging the two myograph levers to move in the plane of the diaphragm of the microscope ocular, all four images are arranged to play upon the one camera plate.

This system can be used for recording the combined action-currents and mechanical contraction of two muscles concurrently, or, if so required, can be used for one single muscle. The galvanometer leads are silver-silver chloride pins, arranged one in the belly of the muscle and one in or near the tendon. Where the action-currents were obtained from two neighbouring muscles concurrently, it was found that provided the tendon leads were in a part freed from underlying structures, and the other leads piercing the surface of the muscle, where not only the skin and underlying connective tissue had been removed but also the surface of the muscle substance carefully cleaned of investing perimysium, no transmission from one muscle to another occurred. The independence of the leads was quite easily demonstrated by the presence of large deflections in each during periods of quiet in the other. Provided these precautions are taken, the transmission of action-currents from one muscle to the leads from another, as described by Forbes and Barbeau (10), does not occur. The time records and signal records have been obtained in the manner described in earlier papers

Taps upon the muscle tendon to elicit tendon jerks have been made by the finger, by a rubber-covered rod, by a hinged tapper arranged so that the second blade falls a little later than the first, or by a rotary cam arrangement, which releases a spring lever which bounces back from another spring and is caught by the higher level of the revolving cam on the return, so delivering a tap of extremely brief duration.

Results.

Isometric records of tendon jerks in quadriceps (11 and 2) and in supraspinatus (7) are usually obtained with the muscle in a state of preceding quiescence. In an extensor muscle in a decerebrate animal this means slackening of the muscle until no stretch reflex is present, for, as Liddell and Sherrington (20) described, the stretch of these muscles beyond a certain length evokes in the muscle stretched a reflex contraction, the stretch reflex, which is maintained during the maintenance of the imposed stretch. A tendon tap upon the quiescent muscle elicits the conventional tendon jerk, with its action-current, followed by a "hump" with its action-current. The silence in the string described by Fulton and Pi-Suñer is seen only when a background of stretch reflex exists. Under these conditions it has been the experience of the author that, provided no mechanical vibrations are allowed to affect the galvanometer leads, this silence in the string record is always demonstrable.

Tendon jerks during a mild degree of stretch reflex are seen in Plate 7, fig. 1, in vastus internus and crureus combined (the remainder of quadriceps having been paralysed and resected). The action-currents of crureus and vastus internus are recorded separately and concurrently in two strings, and it can be seen that the efferent wave of the tendon jerk appears in each, and also the "silent period" (as it will be called for brevity) in the string record. It happens that wherever the galvanometer leads are placed in the muscle or muscle group exhibiting the jerk, the action-currents always show the silent period, and, therefore, the action-currents have ceased throughout the muscle group during this period.

The silent period is not the result of the mechanical strain of the tap upon active muscle elements, because in deafferented muscle similar or heavier tendon taps (Plate 8, fig. 19, B) are without effect upon the action-currents of a contraction, and a slight mechanical fall following the tap, owing to the peripheral mechanical effect described by Gasser and Hill (13), is the only sign of any disturbance of the muscle. The silent period of the tendon jerk therefore requires an intact afferent supply to the muscle for its appearance. It accompanies tendon jerks elicited by tapping the table with a rubber-covered rod, and therefore is in no sense an electrical artefact dependent upon conduction away from the muscle.

The muscle therefore receives no exciting volleys during the silent period, and the absence of mechanical relaxation must therefore be due to the persistence of the mechanical response set up by the preceding efferent volley of the tendon jerk, which lasts long enough to maintain the contractile tension of the muscle until the silent period has finished and reflex reactivation of the muscle ensues. The shortness of the silent period thus enables the duration of the already-initiated twitch contraction to bridge across the interval of inhibitory interruption.

The variation in the duration of muscle twitches in the same muscle from animal to animal will be described in another paper to be published shortly, but it is evident here that if the muscle were to show tendon jerks of short enough duration, relative to the duration of the silent period, then mechanical relaxation should occur during that period. This can, in fact, occur, and in fig. 2 the tendon jerks in a quadriceps which was very rapid indeed show a fall after each rapid jerk, and this fall ceases only when recovery from the silent period occurs. The silent period can be definitely regarded, then, as a cessation of discharge during the tendon jerk, and occurs throughout the muscle exhibiting the jerk.

Here, however, another question is raised. How is it that the muscle in fig. 2 is able to relax so far if relaxation *per se* causes the recovery from the silent period ? Indeed, in the first jerk in fig. 1, the recovery from the silent period takes place before any relaxation occurs, and the " hump " or " myotatic appendage " is grafted on to the jerk plateau. Relaxation here seems not to be necessary, just as in fig. 2 it seemed without effect. The point may be further examined in the muscles which extend the ankle, gastrocnemius and soleus, which present the advantage of having twitch durations particularly favourable for examination of the question. That is, it can be taken for the present for granted that gastrocnemius responds with a peripheral contraction duration, which is rapid and uniformly so, while soleus responds with slow peripheral duration which has no rapid element in it. Quadriceps, on the other hand, is a muscle of mixed elements of slow, intermediate, and rapid, duration, and it is often impossible to say whether a given relaxation is occurring in one element or another. In gastrocnemius at moderate initial tension (*i.e.*, short length) and consequently showing but little stretch reflex (fig. 3) taps upon the tendon elicit the conventional tendon jerk with action current, twitch-like corresponding contraction, silent period and hump

Lengthening the muscle further (figs. 4 and 5) causes further increments of stretch reflex and a more intense action-current background, and now tendon taps (or table taps) cause jerks of smaller mechanical tension but with the same size of action current Further increase in the stretch reflex background has the effect of abolishing the mechanical jerk completely, although the action-current and silent period remain. A stage is reached where the stretch reflex background is so well developed that only a small efferent volley, and usually an exceedingly short silent period, remain (fig. 6, from another preparation). The silent period is sometimes absent after a very small volley. A silent period is never seen without an efferent wave of certain size.

With soleus, where the muscle response is of long duration, the "hump" tends to occur at the angle, even at very low initial tension (fig. 7), and as long as the mechanical jerk is marked the silent period remains much the same length. In this muscle it often happens that a very slight degree of stretch reflex abolishes the mechanical jerk response and only the efferent volley and silent period remain (figs. 8, 9 and 10). Here again the efferent volley always accompanies the silent period.

Cooper, Denny-Brown and Sherrington (6) found that the flexor units available to one ipsilateral afferent nerve in the spinal cat included some units which could be excited from another afferent nerve and some which were often not excited by most other afferent nerves. The exciting effect of one afferent source could be occluded at the overlapped units by the excitation of the other afferent source. So, too, in the present case the fact that the mechanical tendon jerk is swallowed by a stretch reflex is evidence of the fact that the tendon jerk units are already activated by the stretch reflex. The defect is not in the end organ appreciating the tendon tap, for the action-current and silent period still exist, and it does not mean that the effector units are excluded from excitation by the tendon afferents, for the efferent volley still exists; therefore, the defect of the mechanical jerk must lie in the muscle, and a ready explanation of it lies in the fact that the muscle units are already being activated by a tetanic discharge, but one which is slow enough not to leave many units refractory at one instant and yet rapid enough to prevent one more impulse from adding any mechanical tension. The units activated by the stretch reflex are probably therefore activated by a slow series of impulses, just sufficient to fuse the mechanical result, and this is confirmed by investigations by a more direct method, which will be reported in a later paper. Viewed in this light the occurrence of the action-current of the tendon jerk at almost full size, when the mechanical response is absent and growing smaller as the background reflex is further increased in intensity (rate of discharge increasing), is comprehensible.

The silent period is therefore an accompaniment of the efferent volley of the jerk, provided that volley is of any appreciable size. From the total cessation of action-currents it would seem that this period is, in fact, an inhibition. That this is the case is seen when the jerk is played against an excitatory reflex, such as crossed extension (figs. 11 and 12), where it grows shorter as the excitation recruits and longer again in the after-discharge. So also the silent period appears against a background of reflex stepping or ipsilateral extension, always appearing as a complete cessation of all action-currents following the efferent volley of the jerk.

The effect of permitted shortening during the contraction of the jerk response is stressed by Fulton and Pi-Suñer as evidence of the dependence of the appearance of the "hump" upon the amount of lengthening of the muscle in relaxation. In fig. 13 (Plate 8) the shortening occurring during the jerk is small (1·17 mm. per kilo.), while in fig. 14 it is large (4·83 mm. per kilo.). The two jerks were obtained from the same muscle within two minutes of each other and begin from the same reflex tension. The duration in fig. 13 is 88 σ and the jerk tension is 200 grams, while in fig. 14 the duration is 69 σ and the tension 180 grams. This difference is probably solely due to the shortening, and has been obtained often in mammalian muscle-nerve preparations. The silent period, however, is the same length in each, although the shortening in the former is only 0·23 mm. and in the latter 0·86 mm. The time of occurrence of the "hump," therefore, is only altered in relation to the angle, and not in relation to the duration of the silent period, which preserves some degree of constancy at a given reflex tension.

To determine whether the silent period was due to the tendon tap or otherwise, the tendon jerk was imitated by applying a break shock to one ventral root in continuity, *i.e.*, causing a single synchronous efferent volley during the course of a stretch reflex. This was repeated during varying stretch reflex tensions, and with different efferent ventral roots, and compared with the result of a tendon tap under the same conditions. It was found that the efferent volley alone produced a silent period following it, indistinguishable from that accompanying a tendon jerk at the same tension, and, conformably with the silent period of the jerk, all discharge ceased for that period, although only one ventral root was stimulated (figs. 15 and 16). It was possible to avoid escape of the stimulus to the accompanying dorsal root and the neighbouring ventral roots. Especially is this avoidance attainable, with certainty, and without much difficulty, in the case of the long seventh lumbar ventral root.

The silent period, then, is an accompaniment of—or, strictly speaking, an immediate sequel to—a volley of motor impulses however produced, and as has been seen from the study of the tendon jerk, the efferent volley must be of some size—a *large* relatively *synchronous* volley is necessary to produce it. This means that an efferent wave must occur in a certain number of motor fibres at approximately the same time for the inhibition to occur.

The next step is to determine whether activation of purely motor units occasions the appearance of the silent period; it was shown above that a tendon tap in deafferented muscle does not cause it, yet it might occur after a synchronous volley in the motor units to deafferented muscle. In a decerebrate cat a muscle which had had its afferent supply interrupted by section of the afferent roots supplying it (under deep anæsthesia and with strict asepsis) four days earlier was activated by break shock during the course of reflex responses. As is shown in figs. 17, 18 and 19, the break shock, which alone causes a muscle twitch, causes a smaller action-current when it is superimposed upon a reflex discharge. The action-current is smaller the greater is the intensity of reflex discharge upon which it falls, and this can be interpreted as being the result of a greater number of motor fibres being in some part of a refractory phase, owing to the greater number of nerve fibres in activity, and also probably a greater rate of discharge in each nerve fibre as the reflex develops.

It is found that when the discharge is small the efferent wave caused by the break shock is followed by a silence of some 40 σ, and when the background is intense the same period of silence occurs, but it is distinctly relative silence, *i.e.*, it is broken by small waves. In fact, the silence is never actually complete as is the silent period of the jerk, except when all fibres are activated by the break shock (an occasion indicated by an action-current the same size as that of the control, with no background). From other considerations the discharge in a deafferented crossed excitation, such as occurs in figs. 17 and 18, is following the rate of the stimulus (50 a second). Therefore, it is evident that the break-shock activation has thrown the units activated into some kind of refractory phase, and the ripples which escape the refractory phase can be regarded as the continuity of reflex discharge in units which were refractory at the moment of application of the break shock. Presumably, the units which were excited were subjected to a propagated disturbance, which travelled centralwards and set up this refractory phase in the central portion of the unit, for the local refractory phase in this nerve never exceeds 4 σ in my experience. The central disturbance may have been an inhibition set in action through the axon collaterals of Golgi, but in that case it did not affect any motor units besides those activated, nor those of any other muscle examined. The activation of the motor units of the deafferented flexor did not affect the motor discharge of the extensor for example, or the units of one extensor affect those of another. Therefore, it would seem desirable to consider this type of silence as a central refractory phase. This refractory period is a little shorter for more rapid muscles.

There is, however, a great difference between the central refractory phase and the silent period of the tendon jerk. The former affects only the units *activated and at its longest is comparatively short-lasting. The latter affects* all the muscle centre, even though only a few units be activated, and can last very much longer with comparable degrees of background. Furthermore, the silent period can affect not only the motor units of the muscle where the jerk occurs, but in favourable conditions can appear without the efferent accompaniment in other muscles of the extensor centre. In fig. 20 it is seen in gastrocnemius during a tendon jerk in quadriceps. The latency of the appearance of the silence in gastrocnemius was 26·6 σ, 24·0 σ, and 18·6 σ, in three instances where no efferent (jerk) wave preceded it, measured from the onset of the tap upon the tendon in quadriceps. The latency of the tendon-jerk efferent volley in this quadriceps averaged 6·4 σ, from records of jerks with the string action current from quadriceps, while that of gastrocnemius averaged 8·6 σ. It will be seen, therefore, that there was sufficient time for the efferent volley of the tendon jerk in quadriceps to set up some afferent discharge in the muscle which would cause inhibition of the units for gastrocnemius. The earliest computed time for cessation of action-currents in gastrocnemius (assuming that the wave in each case travels as rapidly as do the volleys of the tendon jerk) is some 15 σ after the tap on the tendon of quadriceps.

It seems certain, therefore, that the silent period of the tendon jerk and the break shock to the ventral root is a reflected inhibition from the excitation of the muscle. Though a great many records have been made, it has never once been seen to occur when no efferent volley occurred, and it must be the efferent volley which sets up the return inhibitory afferent volley. A tendon tap which does not cause a jerk efferent volley never causes a silent period.

The silent period is seen in the tendon jerks of the spinal animal, and the "hump" is evident, especially in the extensors of the dog after section of the cord some months previously. In the decapitate cat a tap upon the tendon of tibialis anticus (flexor) often elicits a jerk of a latency averaging 8·5 σ and often showing a small action-current preceding a small "hump" on the decline. These tendon jerks never produce an accompanying jerk in gastrocnemius or soleus unless the mechanical tap be sufficient to cause a direct jerk in these muscles. It was found, however, that a tap upon the tendon of gastrocnemius in the decapitate preparation, besides causing a jerk with a "hump" in that muscle itself, often caused a small jerk-like twitch in tibialis anticus, of a latency constantly very long (22·7 σ from the onset of the tap in gastrocnemius, fig. 21), and it is thought that this disturbance is the reciprocal of the onset of the silent period in the extensor where the jerk is taking place.

The flexor in the decerebrate animal shows a short but definite silent period, which can abolish volleys in a flexor excitation and is well marked in after-discharge (fig. 22). It will be remarked that the efferent volley in this case is much larger when the tap occurs upon a background of flexor excitation than when it occurs alone, and this means undoubtedly that the flexor afferents which convey impulses tending to excite the same muscle overlap among themselves much less than do the extensor afferents of similar type. This means that these flexor-exciting proprioceptive afferents can exert their effect much more readily with an excitation summation already proceeding at the motoneurones. Since the efferent volley is then larger, the silent period of the flexor jerk is then also larger.

In the decerebrate cat a tendon jerk which sets up clonus (fig. 23) differs from the tonic tendon jerk only in the synchronous nature of the wave which normally causes the "hump," and this synchronous wave is then in turn followed by a silent period and another synchronous recovery. The succeeding beat here, as in the case with the "hump" of the ordinary jerk, is not caused by the relaxation of the mechanical contraction, for if the example shown in fig. 23 be carefully examined, the first clonic beat has its action-current beginning exactly at the angle of the jerk, and so also the 3rd, 4th and 5th action-currents, while the 6th appears 2·8 σ before the angle of the preceding mechanical response, and the 7th 6·1 σ before the angle. The time of occurrence of these succeeding volleys is dependent upon the co-existing degree of tonic-stretch reflex, in the same way that the "hump" of the tonic tendon jerk occurs earlier when the co-existing stretch reflex is greater.

Discussion.

The evidence which is presented suggests that any motor discharge which occurs at approximately the same time in a sufficient number of motor fibres sets up a return afferent influence, which has a deep inhibitory effect over all the motor units of the muscle concerned and a slight inhibitory effect over the units of muscles of related function, and also in the case of extensors a tendency to excite the units of the opposing flexor. There must be, therefore, an afferent end organ in muscle which can appreciate excitation of muscle. Since the discharge of all other units has ceased by the time the action-current of the excitation causing the inhibition has itself reached complete equilibrium, it follows that either the inhibition is extremely rapid in origin and effect, or else that the action-current causing the disturbance has left all the motor units in a refractory state with which the inhibition overlaps. Since in the case of break-shock excitation of one ventral root supplying the muscle the latter is certainly not the case, and there is good reason to believe that the tendon jerk efferent volley involves only a fraction of the motor units, the former seems the only possible explanation.

The end organ which subserves the appreciation of muscle *excitation* must therefore be capable of appreciating the onset of the electrical wave which precedes contraction, and must be provided with an afferent fibre which can conduct very rapidly. There is such an end organ in the annulospiral ending of Ruffini—the broad ribboned end of an afferent fibre which is among the largest of nerve fibres, applied in such a manner as to embrace the greatest possible surface of a muscle fibre. The muscle fibre under the annulospiral ending is specialised to a degree, often appearing as a mass of nuclei separated from the nerve ending by a membrane which is extremely thin. The afferent nature of the ending was proved by Sherrington (22), and the muscle fibre has been shown by Boeke (3) and Hinsey (15) by degeneration experiments to possess a motor nerve ending. Such is the main ending of the muscle spindle; whether the remaining endings in the muscle spindle are of similar function it is impossible to deduce at the present.

The end organ for the appreciation of tension, whether active or passive, is seen as the tendon organ of Golgi, supplied by a similar large afferent fibre and scattered under the aponeuroses of insertion. As Ruffini (21) showed, the fibre innervating the tendon organ often supplies a modified Pacini corpuscle, and so the reflex significance of the two would appear to be the same.

Adrian and Zottermann (1) described the discharge in the afferent fibres in the nerve to the M. sterno-cutaneus of the frog resulting from stretch of the muscle, and succeeded in isolating one muscle end organ responding to stretch tension. If the present deductions are true, then a simple muscle spindle should respond only to muscle excitation and not to stretch, and therefore it seems possible that the end organ responding thus to stretch is of the primitive tendon afferent type, of which, according to the drawing of Kölliker (19), there are at least four in that muscle.

It seems likely that the individual afferent fibres from a given muscle overlap with one another in their representation at any one motoneurone of that muscle in the same way that different afferent nerves overlap in their effects or occlude each other upon a group of motoneurones accessible to them all. By this it is meant that any one afferent fibre, from a tendon organ for instance, by branching can affect all the motoneurones for that muscle. Some it can affect more than others, and it is fairly certain that by one impulse alone it can discharge none of them. This is apparent when it is considered that the tap which elicits the tendon jerk does so in virtue of its synchronous discharge of all tension afferents. In an acute spinal preparation a maintained stretch produces no maintained stretch reflex, and yet there is no reason why the tension end organs should be more adaptable in a spinal animal than in a decerebrate preparation. But if the discharge be a synchronous one, such as is caused by a sharp tendon tap, then each can summate with the other before central dispersion occurs, and the tendon jerk results. This is especially the case when a tap upon the table, causing but a faint jar of the muscle, must act only by throwing all the tendon organs into discharge at the same instant (figs. 4 and 5) and the efferent volley of the "jerk" ensues.

So, too, the muscle spindles which sample the efferent discharge throughout the muscle can completely inhibit the motor centre if a sufficient number of them discharge in synchronism, and this is determined only by an efferent motor wave of sufficient synchronism in a sufficient number of units. Thus it happens that a tendon jerk by its synchronous efferent volley determines the appearance of the silent period, which is only the effect of the resulting synchronous inhibitory volley. The muscle spindles, however, must keep up their asynchronous discharge during any asynchronous efferent discharge, and their effect then, although not sufficient to cause complete inhibition of all units at any one moment, yet will summate algebraically with the state of excitation at any one locus. The sum total of the effect should therefore be a *damping* of the discharge at the motor units and a resulting slow, relatively non-fatigable, rate of discharge, unless the excitation at the unit completely overwhelms the inhibition. The transition from synchronous to asynchronous discharge is well seen in the transition from clonus to tonic posture after the tendon jerk in fig. 23.

This conception of the nature of the tendon jerk involves the consideration of the tendon jerk as a single efferent volley (always slightly asynchronous, and more so in the spinal preparation). Fig. 24 shows how asynchronous the efferent volley of the decerebrate jerk may become. In quadriceps the relative durations of tendon jerks cannot be relied upon for information as to the number of volleys in the jerk, because the duration varies according to the fibres in which it occurs. The tendon jerk in soleus in the decerebrate preparation is certainly never more than one impulse for one tap, and the effects of two successive taps are two impulses only if the two taps are more than 100 σ apart, a value which corresponds with the "refractory period" of the tendon jerk found by Dodge (8), Golla and Hettwer (14). Hoffmann (17), and Strughold (26).

The silent period follows the twitch produced by a break shock to the intact muscle nerve, being then the result of direct stimulation of the inhibitory afferents overlapping with the true refractory period. This type of silent period has been described by Hoffmann (16) as the refractory period of the centre. The conception presented in this paper further entails that the rate of clonus is the true rate of discharge of the motor unit under those conditions and represents the rate of discharge in any particular unit in a tonic contraction of the same tension in the same muscle. (The rate of discharge in flexors, being less damped, is considerably higher.) Further evidence for these rates of discharge from more direct sources will be dealt with in a later communication.

The lengthening reaction appears to be due to the repercussed inhibition from the muscle spindles, ensuing upon the increased excitation predominating during the first part of the applied stretch, and the shortening reaction the reassertion of the tendon excitation, after the accumulated inhibition from discharge at a greater rate (at the former greater length) had passed off. It is interesting in this connection that in preparations which show a silent period easily transmitted from one extensor to another, a stretch of one of these extensors evoking in it a stretch reflex and then a lengthening reaction, causes partial or even complete inhibition of a stretch reflex present in another. Although it was not looked for (these preparations had the opposite limb immobilised) the crossed extensor contraction of Phillipson would have occurred at the same time. Sherrington (23) deduced the presence of such an inhibition when he first described the lengthening and shortening reactions.

Since the silent period can be broken down by excitation most easily at its latter end and is found most intense in its early part, there is reason to believe that one wave of excitation produces only one afferent wave of inhibiting impulses with a most intense effect upon its arrival. This means that in the ordinary condition of reflex posture a continuous stream of impulses at fairly high rate is coming from the tension end organ and two series at relatively slow rate are occurring, one of them proceeding down the motor nerve fibres and the other proceeding up the afferent fibres from the muscle spindles. From the rapid diminution in duration of the silent period caused by excitation of the motor fibres at a high rate of repetitive stimulus, it would seem that the muscle spindle is relatively rapidly adapted to (or fatigued by) a rate of excitation which is more rapid than usual, and this is probably the reason for the involuntary tonic abduction of the arm after long-continued forceful effort at producing abduction ("Katatonischer Versuch" of Kohnstamm (18)).

If the deductions concerning the function of the muscle spindle made here are true, then the observation of Byrnes (5) that in paralysis agitans the muscle spindles of affected muscles are degenerated while the tendon organs are intact, accounts for the rigidity occurring in that disease, for then the motor units are undamped in their discharge when excited from any source. The rigidity could therefore be due to a peripheral selective effect with local action, and no other pathological alteration need have any effect in this connection.

Summary of Conclusions.

1. The complete absence of action-currents during the tendon jerk is due to an inhibition caused by the excitation wave of the jerk.

2. The appearance of this silent period is probably merely the effect of the synchronism of inhibitory impulses which in normal circumstances of discharge, other than clonus, are asynchronous. This proprioceptive inhibition is a sequel to any excitation of the muscle.

3. The muscle spindles are regarded as the end organs in which the inhibitory impulses are set up, and the tendon organs of Golgi as the organs which appreciate tension, whether passive or active.

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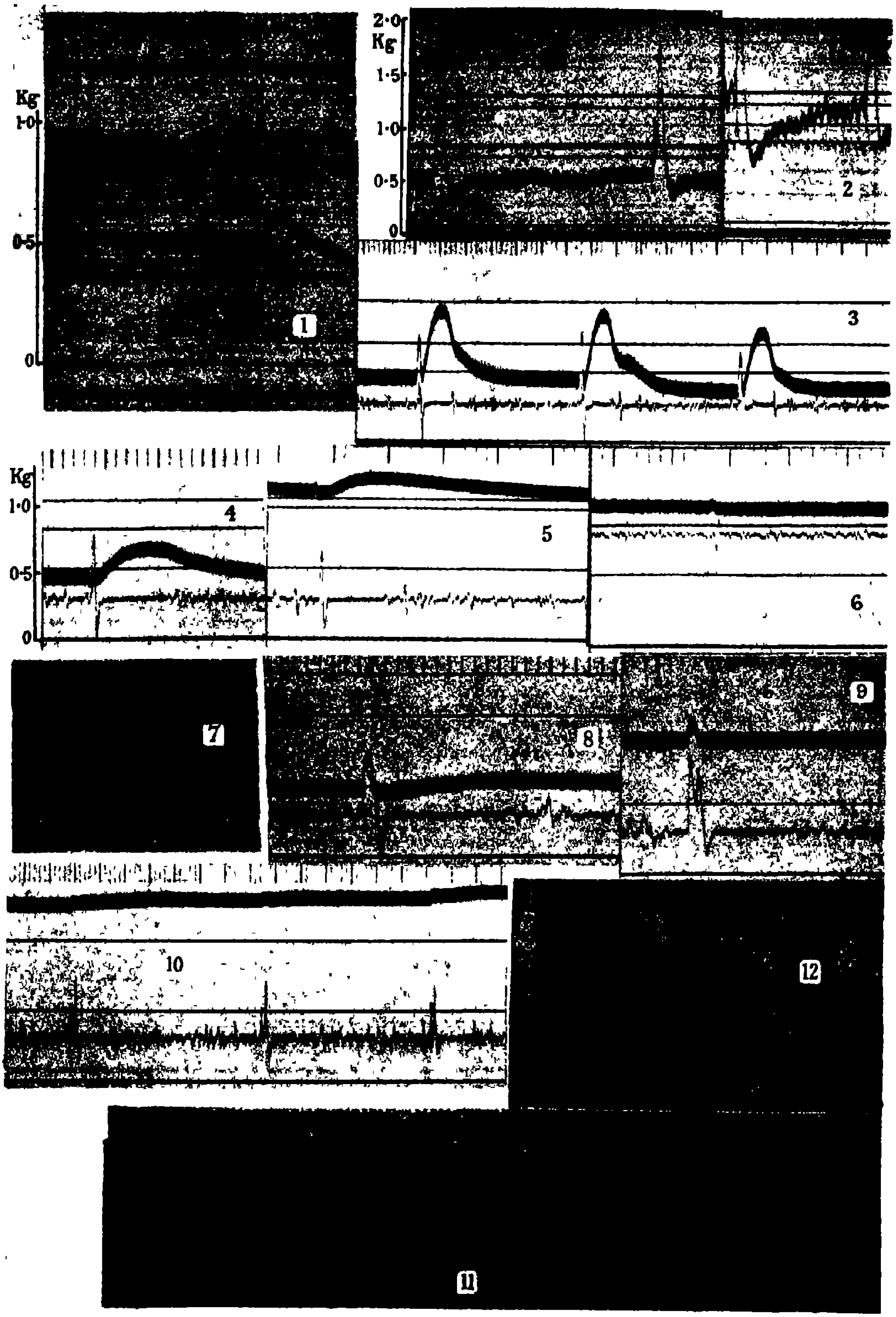

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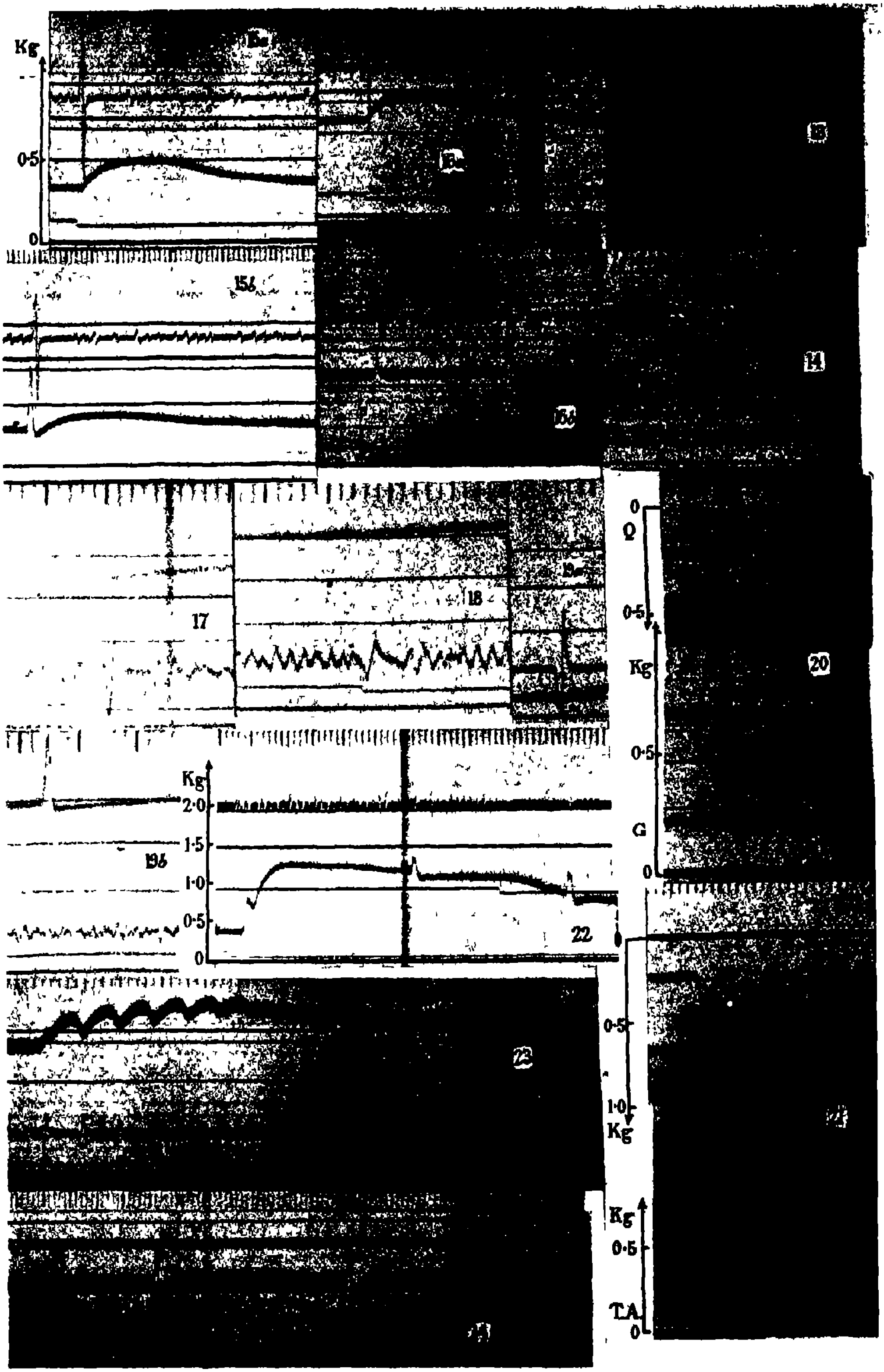
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DESCRIPTION OF PLATES

All figures read from left to right In records from a single myograph an upward movement indicates a rise in tension (contraction) a downward movement means a fall in tension (relaxation) Where two myographs are recording together the upper tracing moves downward for contraction and upward for relaxation while the lower myograph moves as usual The sharp peak in the myograph record is the record of the tap upon the muscle tendon and is the direct result of the sudden rise and fall of tension produced by the impact of the tap The vertical strokes at the top of each record are produced by the spokes of the time marker (Rayleigh wheel) and occur at 1/50 second intervals each 1/10 second being stressed by a slightly thicker spoke In all the preparations called decerebrate the plane of brain stem section is intercollicular

PLATE 7

FIG 1 —M Vastus Internus and Crureus together recording on one myograph Decerebrate preparation Taps upon the tendon during a slight stretch reflex The leads for the galvanometer string near the time marker lines are in crureus The action currents below the base line are led from vastus internus Remainder of M Quadriceps resected.

FIG 2 —M Quadriceps Decerebrate preparation The rapid component of this muscle was much more rapid than usual and the tendon jerk was only 24 σ in duration The record in *b* is at higher tension than *a* (within the critical clonic tension) and shows the drop during the silent period more clearly

FIG 3 —M. Gastrocnemius Decerebrate preparation Tap upon the tendon during a slight degree of stretch reflex Note the well marked hump Tension scale as in fig 1

FIG 4 —The same gastrocnemius as in 3 at a greater length Tap is upon the table and the effect is a faint jar to the tendon (throwing all the tension afferent impulses into phase at that moment)

FIG 5 —The same muscle at a still greater length Tension scale as in fig 4

FIG 6 —M Gastrocnemius From another decerebrate preparation Showing a degree of stretch reflex which in this preparation left the tendon jerk only as a very small volley indeed Tap on the tendon Tension scale as in fig 1

FIG 7 —M Soleus Decerebrate preparation Tap on tendon Tension scale as in fig 1

FIG 8 —The same as fig 7 but the initial stretch reflex background increased by increasing the length of the muscle Tension scale as in fig 1

FIG 9 —As fig 8 length still greater Tension scale as in fig 1

FIG 10 —As figs 8 and 9 Still greater length Tension scale as in fig 1

FIG 11 —Taps upon the tendon of soleus before and during an excitation from the peroneal nerve of the opposite side and one tap during the after discharge Decerebrate preparation Tension scale as in fig 4

FIG 12 —Taps upon the tendon of soleus during the plateau of a crossed excitation Decerebrate Tension scale as in fig 1

PLATE 8.

FIG. 13.—Quadriceps. Decerebrate preparation. Tap upon tendon. Tension scale as in fig. 1.

FIG. 14.—The same muscle as in fig. 13 about two minutes later. By means of a spring inserted between the hook and the myograph the shortening during contraction is much increased. See text. Tension scale as in fig. 1.

FIG. 15.—Soleus. Decerebrate preparation. Slight stretch reflex. All nerve roots (ventral and dorsal) intact. A break shock is applied to the seventh lumbar ventral root in A. In B is a tendon jerk at the same reflex tension a few seconds later.

FIG. 16.—The same muscle as in fig. 15 and the same procedure, but the effect is compared at higher initial tensions of stretch reflex. Tension scale as in fig. 15.

FIG. 17.—M. Soleus. Completely deafferented for four days. Excitation of the crossed sciatic causes the background discharge. A break shock to the small nerve to soleus occurs at the fall of the signal. Tension scale as in fig. 4.

FIG. 18.—The same as fig. 17, but the break shock falls later when the crossed excitation is reaching its full development. Tension scale as in fig. 4.

FIG. 19A.—The same as figs. 17 and 18. No crossed excitation and the control of the break shock effect alone is seen.

FIG. 19B.—As in fig. 18, but taps upon the tendon. Tension scale as in fig. 4.

FIG. 20.—Below is the myograph tracing of M. Gastrocnemius in tonic stretch reflex contraction with the accompanying action-current record. Above is the myograph record of M. Quadriceps (pulling round the patellar groove). A tap upon the tendon of quadriceps causes in it a jerk and in gastrocnemius a cessation of action-currents. Decerebrate preparation.

FIG. 21.—M. Gastrocnemius and Soleus above. M. Tibialis Anticus below. Each has with it a galvanometer string recording its action currents. A tendon jerk in the extensor causes a delayed twitch in the flexor. Decapitate preparation.

FIG. 22.—M. Tibialis Anticus. Decerebrate preparation. Taps upon the tendon during the course of a flexion reflex.

FIG. 23.—M. Quadriceps. A tap upon the tendon sets up a clonic jerk. Decerebrate preparation. Tension scale as in fig. 1.

FIG. 24.—M. Soleus. Decerebrate preparation. Taps upon the tendon during a stretch reflex. Note the two small action-currents of the first jerk and compare them with the single large action-current of the second jerk. This is the maximum degree of asynchronism of the efferent volley seen in decerebrate jerks, and it is reasonable to assume from the small size of each member of the small double volley that each muscle fibre still receives but one impulse. Tension scale as in fig. 1.

The Anatomy and Phylogeny of Spondylus, with a particular reference to the Lamellibranch Nervous System.

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A study of the anatomy of the rather rare Lamellibranch, *Spondylus*, has always attracted the author, owing to the fact that at a comparatively early date the striking resemblance between the eyes of this creature and the remarkable eyes of the genus *Pecten* had been noted. In structure the eyes of *Pecten* stand practically alone amongst invertebrate visual organs, and since, by reason of their complexity as well as number, their evolution has been associated with the faculty of swimming, it was a matter of considerable interest to determine the relationships of *Pecten* with *Spondylus*, for whilst *Pecten* can swim, *Spondylus* lives attached to rocks.

The investigation was made purely from the above view-point, and hence minute details of histology, etc., have not been given. Resemblances to *Pecten* were expected. One very unexpected morphological anomaly turned up, however; and in the midst of a remarkable agreement with the genus *Pecten*, one system, the nervous system, presented a condition altogether unique amongst Lamellibranchs. To obviate repetition, reference should be made to an account of the anatomy of the genus *Pecten* by the author (Dakin, 1909) and also to a paper, following this, which deals with the eye.

As pointed out by Ridewood (1903), the Lamellibranchiata have ever been a most troublesome group to classify; witness the inadequacy of differences in such features as the shell, hinge, teeth, ligament, pallial sinus, siphons, adductor muscles, etc., all of which have been utilised in attempts at classification. Ridewood adopted, with some modifications dependent on his own extensive work, the classification of Pelseneer, based largely upon the gill characters. In this scheme there is a sub-order, Pectinacea (the Order Eleutherorhabda, to which it belongs, is characterised by gill filaments arranged on the two sides of the gill axis; adjacent filaments are held in position by stiff cilia disposed in patches), which contains the families Spondylidæ, Pectinidæ, and Aviculidæ. The two genera *Spondylus* and *Pecten* are therefore closely

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* The author wishes to express his thanks to the Authorities of the Natural History Museum for permission to examine material, and also to the American Natural History Museum, Washington, for gift of a rare specimen.

associated, but *Amussium* is not even put in the same sub-order. The older grouping of Pelseneer, in which the genera *Amussium*, *Spondylus*, *Pecten*, *Chlamys* and *Pedum* are all brought close together, is nearer the truth, but even here *Spondylus* is placed in a different family from *Pecten*, a feature which seems rather unnecessary.

The shell of *Spondylus* is very different from that of *Pecten*. It is well known, some long-spined species of great beauty having been particularly favoured by shell collectors in the past. Other species are more coarse, rough, and with shorter spines. The valves are usually heavier than those of *Pecten*, inequivalve —one projecting considerably dorsal to the hinge line in a very characteristic manner, and frequently one valve is much flatter than the other. In *S. gæderopus*, from the Mediterranean, one valve is definitely flat, the other convex. In *S. Americana* both valves are convex.

Now it is worthy of note (for reasons to be given later) that the *flat* valve, which is the right one in *S. gæderopus*, is the attached valve. *Pecten maximus* has one valve very convex, whilst the other is quite flat; but the animal always rests on the *convex* valve. At a first glance, therefore, the two shells are not only rather unlike, but the orientation of the creatures appears different. The innate identity is realised, however, when one finds that, notwithstanding such diversities in valve shape, all the species of the two genera examined tend to rest on the same valve—the right.

Again, the convex *right* valve of *Pecten maximus* actually overlaps the flat left valve by ⅛–¼ inch; we have noted above that the flat *right* valve of *Spondylus* greatly overlaps the left valve. We have two points indicating fundamental resemblances in orientation to put against differences in shell form. These resemblances become of greater importance when one remembers that there is no rule in the Lamellibranchiata regarding the orientation, and that in one genus the right valve may overlap the left whilst in another it is the left valve which is the larger. In fixed species the fixed valve may be the right valve or the left.

Mantle and General Organisation.

With the removal of one of the valves, the resemblance between the animals, *Spondylus* (fig. 1) and *Pecten*, is seen at a glance. There is the same single and large adductor muscle situated centrally and obviously made up of two parts (histologically different, fig. 1, M.S. and M.N.) and the same type of mantle, the edge of which is so characteristic in the genus *Pecten*, where it is closely correlated with the power of swimming.

The Mantle consists of a pair of thin transparent sheets of tissue bordered by a much thickened muscular edge. The two mantle lobes are attached at the hinge line and free everywhere else, except that the inner layers are confluent a short distance before the hinge line is reached anteriorly and posteriorly. This means that there are no siphons, no fusions of the mantle edge to form inhalant and exhalant apertures, nor any other feature to mark a difference between the anterior and posterior regions. The mantle edge reaches its greatest thickness midventrally, and from this point there is a gradual diminution towards the hinge line.

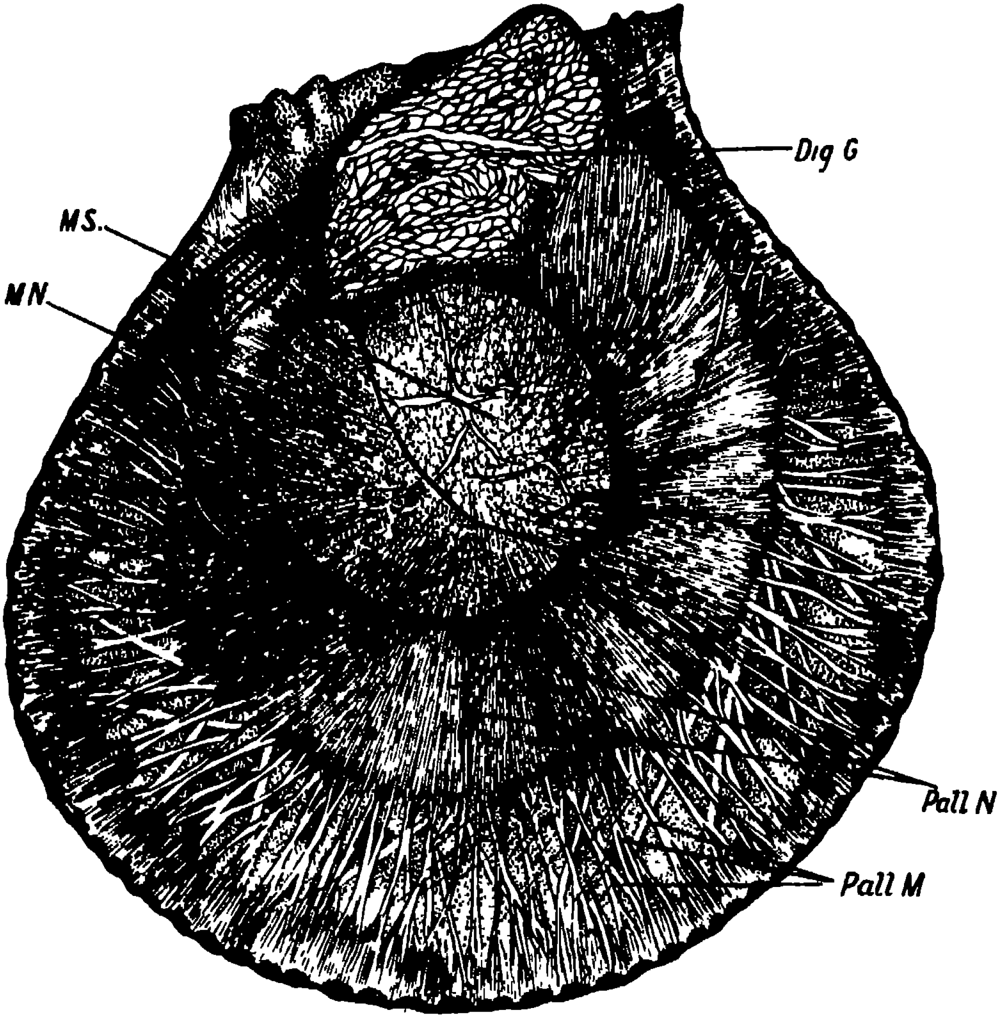

Fig. 1.—*Spondylus gaederopus.* View of right side after removal of right valve of shell. Dig. G., Digestive Gland ; M.N., Non-striated muscle ; M.S., Striated muscle ; Pall. M., Pallial muscles ; Pall. N., Pallial nerves.

The free margin of the mantle presents two well-defined folds; in fact, in section it is Y-shaped. The outermost fold is subdivided, in so far as it bears tentacles in different rows and also a groove from which the Periostracum is secreted. The eyes are borne on this outer fold, on its inner side—see diagram fig. 2—and as in *Pecten* they are largest ventrally and become very small as the hinge line is approached. The innermost fold forms a muscular flap, the Velum. In *Pecten* the velum, together with the adductor muscle, is responsible for swimming. This function is not performed in *Spondylus* although the velum is almost equally well developed. There is one small point of difference between the two. The edge of the velum bears small tentacles in *Pecten*, so that when the shell is opened widely the two "velums" lie with their free edges just touching and the tentacles of each touch or intercross. In *Spondylus* no tentacles have been seen on the free edge of the velum in any of the species examined.

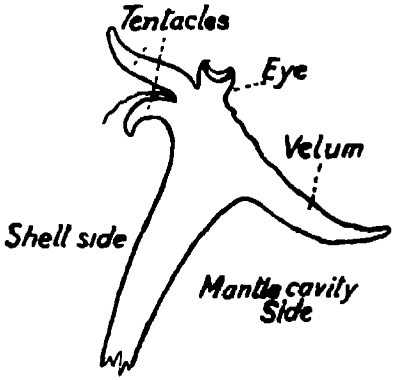

FIG. 2.—Diagram of section through edge of mantle of *Spondylus*.

The Pallial Musculature.

The most important muscles of the mantle are the radial and circular muscles of the mantle edge. The circular muscles lie chiefly in the velum. The radial muscles (fig. 1, *Pall. M.*) attach the mantle margins to the shell valves, and are responsible not only for the general retraction of the mantle when the shell valves are closed, but also for its local movements.

The arrangement of the radial fibres is practically the same as in *Pecten*. They are beautifully fitted for the requirements, and to this end comprise strands whose course is practically perpendicular to the mantle edge and others which run diagonally across the former. The angle of the diagonal muscles changes somewhat as the hinge line is reached (see fig. 1). There is a tendency for the reproductive organs to invade the mantle in the species of *Spondylus* examined. This was most marked in a very old spirit specimen, which had evidently been taken when the animal was mature. All my other specimens had been captured when the gonads were spent, but they gave indications of the same condition. The invasion is not at all extensive.

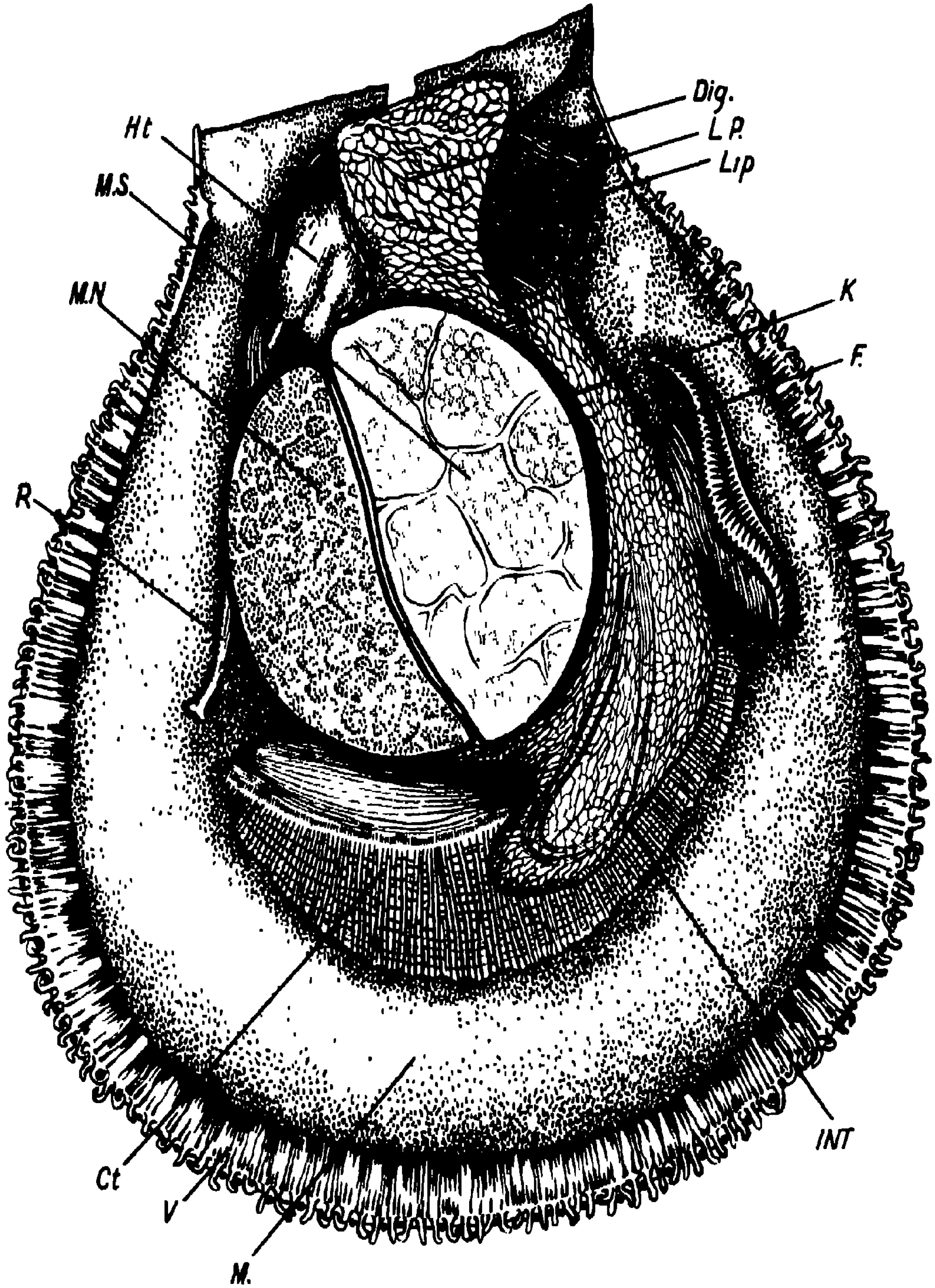

FIG. 3.—*Spondylus gæderopus.* View of right side after removal of right mantle lobe. Ct., Ctenidium; Dig., Digestive gland; F., Foot; Ht., Heart; Int., Intestine; K., Kidney; Lip., Lip; L.P., Labial palp; M., Mantle; M.N., Non-striated muscle; M.S., Striated muscle; R., Rectum; V., Velum.

The Adductor Muscle.

As in *Pecten*, one might describe the organs of *Spondylus* as slung round the single large adductor muscle, which runs across from one valve to the other —eccentrically. It is situated nearer to the posterior margin of the shell. In consequence of this position there is most space left on the dorsal and on the anterior side of the muscle, and it is here that one finds the digestive gland and the visceral mass.

One can see at a glance that, as in *Pecten*, the adductor muscle consists of two parts. These are distinct in both fresh and preserved specimens. The line dividing the two parts runs obliquely, as indicated in the figures, so that one many speak of an anterior and a posterior portion. The fibres of the posterior part (figs. 1 and 3, M.N.) are more opaque—white in fresh specimens —whilst the anterior part is semi-translucent. The interesting feature of the " bipartite " composition of the adductor muscle has already been described in detail for the genus *Pecten*. The translucent fibres are cross-striped, and are alone responsible for the rhythmical contractions which close the valves and make swimming possible. The white opaque fibres do not contract rhythmically, and when the muscle is cut away from the shell they remain in a tonic state and are shorter than the other fibres, which are easily excited by contact. The opaque fibres are responsible for the sustained closure of the shell valves.

Now, just as the eyes and mantle edge of *Spondylus* reveal the ancestry of *Pecten*, so does the adductor muscle. So far as I am aware, no *Spondylus* swims in the adult stages, yet the adductor muscle still presents the two separately specialised sorts of fibres. But there is this interesting point of difference. In *Spondylus*, the volume of the white opaque fibres is relatively greater than that of the translucent fibres when compared with species of *Pecten* The sectional area in *S. americana*, for example, is about one-half that of the translucent portion. In *P. maximus* the area of the opaque fibres is only about a quarter that of the translucent fibres.

Thus the long period during which *Spondylus* has given up its swimming habits has been accompanied by a relative increase in that part of the adductor which is responsible for the ordinary shell closure in *Pecten*, or, putting it the other way, by a decrease of the muscle fibres responsible for rhythmic contractions in that genus. The translucent striped fibres are, however, not only still present, but the volume of this kind of muscle still exceeds that of the unstriped fibres. There are apparently no histological differences between the striped fibres of the two genera.

The large central adductor muscle of *Spondylus* and *Pecten* corresponds to the posterior adductor of those Lamellibranchs possessing two. The anterior has completely disappeared in the adult *Pecten* (although present in the early embryo) and it is altogether absent in adult *Spondylus* also.

Retractor Muscles.

Usually one finds retractor muscles (four in the majority of Lamellibranchs) attached to the foot. In *Pecten* the foot is very small and the retractor muscles have been reduced to one: this retractor is the left posterior. Now, it is interesting that even within the genus *Pecten* signs of still further reduction of this retractor are to be found, for whilst it is moderately developed in *P. opercularis*, it is vestigial in *P. maximus*.

In *Spondylus all* the retractors are missing. This fits in exactly with what might be expected if one assumes that *Spondylus* has evolved from *Pecten*, and that *P. maximus* represents one of the end types in the line of evolution in the Pectinidæ. We shall see that the structure of *Spondylus* can be interpreted perfectly on this hypothesis.

Instead of retractor muscles proper, there are considerable developments of muscle fibres in the tissue of the visceral mass between the foot and the sides of the body and the lips. These muscles run into the mantle

The Foot (fig. 3) is a small organ situated in the same position as in *Pecten*, very high up on the anterior surface of the visceral mass. One often assumes that this small structure is a rather rudimentary organ. The free end, however, negatives this. It is sucker-like, widely expanded, and possesses a peculiar grooved surface. Sections indicate that it has a most extensive nerve supply. Whether these nerves are necessary for the intrinsic muscle fibres of the foot, or whether sensory endings are present in numbers in the epithelium, it is impossible to say at present. It would appear, however, that the foot performs actively some functions, possibly that of cleaning the gills, or as an aid in nutrition Sections failed to reveal any signs of byssal gland in the depths of the foot, and one must therefore conclude that again in this feature *Spondylus* has evolved a stage further along the line already indicated by the different species of *Pecten*.

The pedal ganglia are situated at the base of the foot; statocysts, as they should be called, often present in the foot, are situated as in *Pecten*, *outside* the foot, between its base and the mouth region (*see* later).

Ctenidia.

The Gills or Ctenidia (fig. 3, *Ct.*), structures which have played a very important rôle in the classification of the Lamellibranchiata, are very conspicuous organs. Like the gills of *Pecten* they are slung round the adductor muscle. Each ctenidium consists of a supporting ctenidial axis, from which depend the two lamellæ of filaments. The ctenidium is plicate and heterorhabdic, exactly like the ctenidium of *Pecten*, and so far as morphology and histology is concerned there is close similarity again between the two genera. For example, the principal filaments of *Pecten* bear a curious expansion, which the author called a Respiratory Expansion. It is a plate of tissue with blood vessels running in its margins, and with the intervening surface thrown into folds. The same structure exactly is found on the principal filaments of *Spondylus.*

The "gills" of Lamellibranchs are primarily organs for producing currents which subserve both respiration and nutrition. The mantle lobes are highly vascular, and are the most important respiratory organs. It is difficult, however, to understand how the ctenidia can be refused *all* participation in the function of respiration, as has lately been asserted. At all events, in *Pecten* and *Spondylus* parts of the ctenidia are certainly likely to function in this manner. There are some slight differences between the ctenidia of *Pecten* and *Spondylus*, but they are very minor points, and so far as can be seen differences of the same type exist between species of *Pecten.*

The Alimentary Canal and the Digestive Gland.

The alimentary canal of *Spondylus* is comparatively simple in form and closely resembles that of *Pecten.* The mouth opening is hidden, in the characteristic manner seen in *Pecten*, by the convoluted margins of the lips (fig. 3, *Lip*) and their entanglement. Thus, by the interlocking of the upper and lower lips, the entrance to the mouth is reduced to numerous fine passages The margins of the lips are pigmented with the same orange yellow pigment so characteristic of this region in *Pecten.* The Labial Palps, which are continuations of the lips, are rectangular in shape, with the long side at right angles to the direction of the numerous typical grooves. Details of their ciliation have not been worked out.

From the mouth a short œsophagus leads into the "stomach," which is situated in the midst of the digestive gland. This occupies a position between the adductor muscle and the hinge line, and extends down the visceral mass towards the foot. The intestine (fig. 3) passes through the digestive gland ventrally, and then through the visceral mass to its extreme tip (as in *Pecten maximus* in contradistinction to *P. opercularis*). It then bends abruptly, and returns as an ascending limb close to the adductor muscle, plunging through the digestive gland and leaving it to traverse the pericardium and heart in the manner characteristic of so many Lamellibranchs. No other coiling of the alimentary canal is present, and there is no separate sac for the crystalline style.

Nervous System.

Up to this point the anatomical features indicate the close relationship of *Pecten* and *Spondylus*, and, coupled with certain palæontological data, make it extremely probable that *Spondylus* arose from *Pecten* ancestors. The nervous system, however, presents a real surprise, inasmuch as it breaks away from the general type seen in Lamellibranchs. At the same time, even this can be explained if we derive it from *Pecten* A diagram of the nervous system of *Spondylus*, as seen from a point opposite the foot and thus in the middle line, is given in fig. 4. The usual three lamellibranch ganglia are present—the visceral, the pedal and the cerebral. The visceral ganglia are fused to form a complex ganglion which is very like that of *Pecten* (see figs. 5 and 6). It lies on the adductor muscle just beneath the extreme end of the visceral mass. The complications and the remarkable development of this ganglion have not been generally recognised. Fig. 6 is founded on one of the illustrations in a paper by the present author (Dakin, 1910).

The pedal ganglia are easily found as a pair, close together at the base of the foot, and embedded in connective tissue. The cerebral ganglia are *not* easily seen. They actually lie at the angle of the labial palp and the lips. To make their observation more difficult they are embedded in connective tissue.

The startling feature of the nervous system is obvious at a glance. *The two pedal ganglia are connected to the visceral ganglia. Furthermore, there are no distinct connectives between the cerebral and pedal ganglia.* When the author first saw the nerve on each side running from the pedal ganglion to the visceral he jumped to the conclusion that the former ganglia could not be the pedal at all, because such a connection would be unique in Lamellibranchiata.

Had such a thing occurred in *Teredo* or some other more or less highly modified form it would not have been surprising. To meet it in an animal with such close agreements with *Pecten* was at first disconcerting. In all Lamellibranchs so far studied (and the nervous system is very well known) the rule is to find a pair of cerebral ganglia connected to a pair of pedal ganglia and also connected to a pair of visceral ganglia. This state of affairs is illustrated in the diagram of the nervous system of *Pecten* (fig. 5). It is

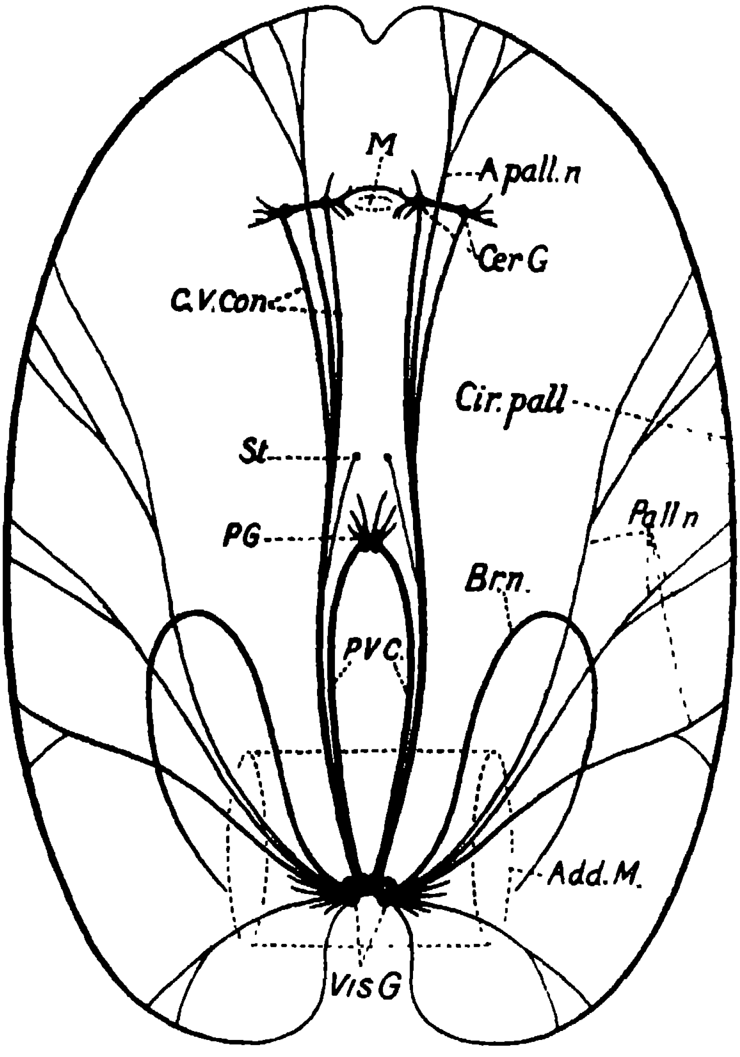

FIG. 4.—*Diagram of Nervous System of Spondylus.* A. pall.n., Anterior pallial nerve, Add.M., Adductor muscle; Br.n., Branchial nerve; C.V.Con., Cerebro-visceral connective; Cer. G., Cerebral Ganglion; Cir. pall., Circum-pallial nerve; M., Mouth; P.G., Pedal Ganglion; P.V.C, Pedo-visceral connectives; Pall. n., Pallial nerves; St., Statocyst; Vis. G., Visceral Ganglion.

perfectly well known to all students who study *Anodon* as a class type and complete their studies later with other typical Lamellibranchs.

There can be no doubt about this extraordinary state of affairs in *Spondylus.* The author has dissected out the pedo-visceral connective in an American species as well as in a considerable number of specimens of the Mediterranean form, *S. gæderopus*. Moreover, this connection between the pedal and visceral has actually been seen once before, but the observer gave no figures, made

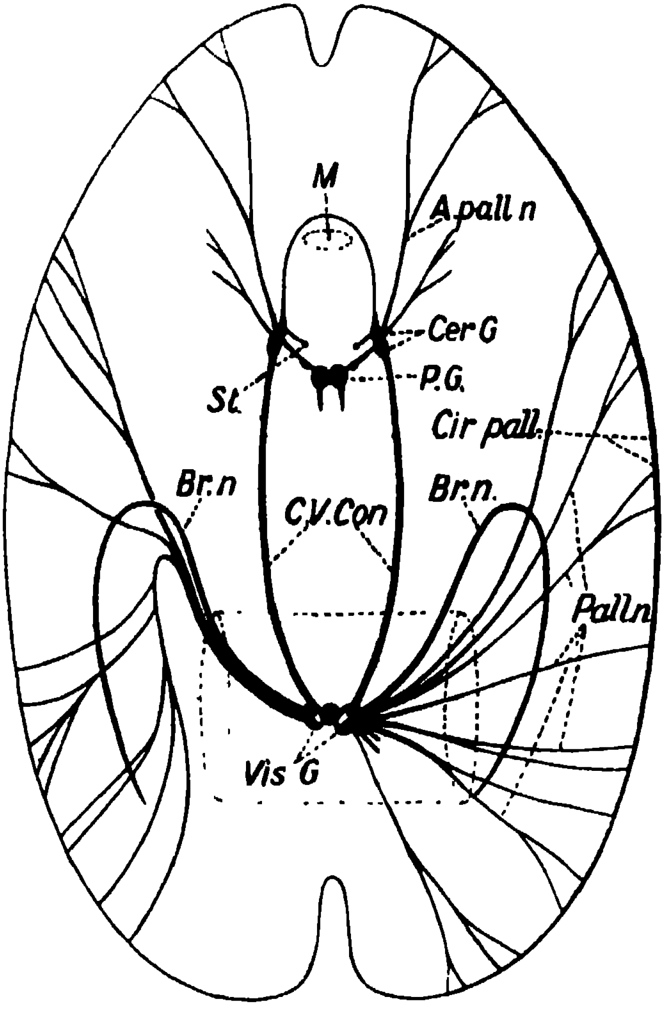

FIG. 5.—*Diagram of Nervous System of Pecten maximus.*—Reference letters the same as for Fi

mistakes in regard to the rest of the nervous system, and misinterpreted the part which was seen (d'Hardiviller, 1893). To obviate any source of error, the present writer cut out from three specimens the whole of the visceral mass, from above the lips down to a point below the foot and extending right across the visceral mass transversely. Serial sections were cut completely through these blocks. It is from these serial sections that the figure (fig. 4) has been constructed. It indicates that in *Spondylus* there are two cerebral ganglia at the sides of the mouth, which are connected by a commissure and by connectives with the visceral ganglion. There are also two ganglia in the foot base, which are connected with the visceral ganglion by nerves which

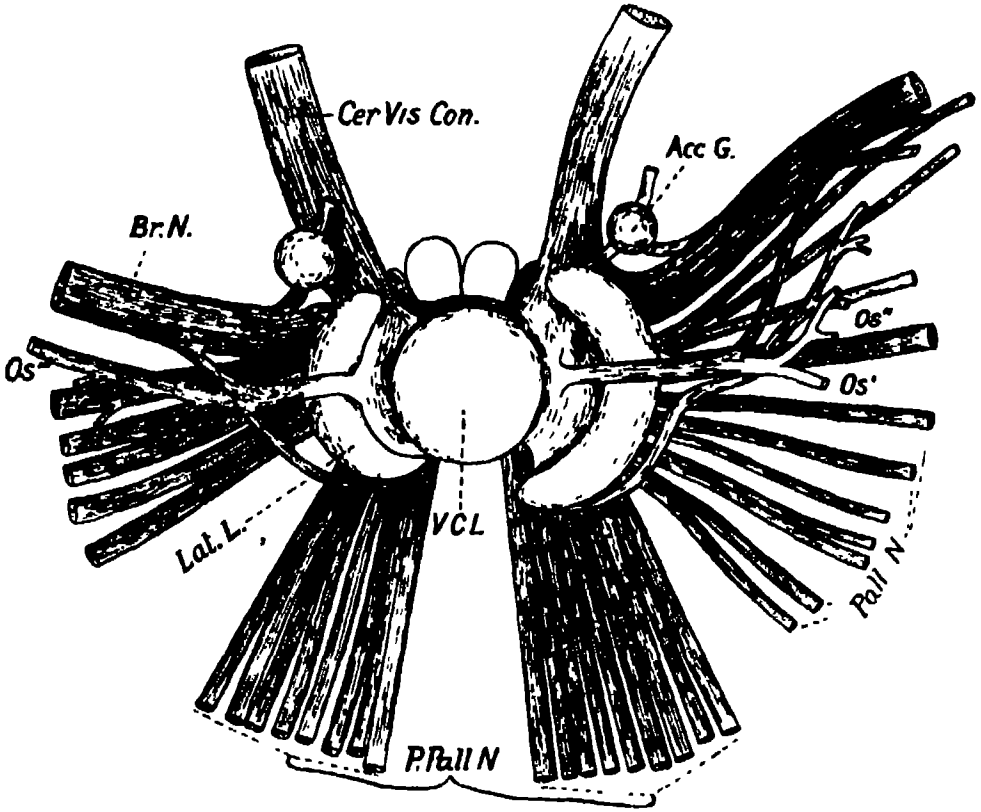

FIG. 6.—*Visceral Ganglion of Pecten maximus.* Acc. G., Accessory Ganglion, Br. N., Branchial Nerve; Cer. Vis. Con., Cerebro-visceral Connective; Lat. L., Lateral Lobe; Os'., Os'', Os'''., Osphradial Nerves; Pall. N., Pallial Nerves; P. Pall. N., Posterior Pallial Nerves; V.C.L., Ventro-central Lobe.

eventually enter this ganglion parallel to and in close juxtaposition with the cerebro-visceral connectives. These ganglia supplying the foot correspond to the pedal ganglia of other Lamellibranchs, although, as indicated below, the author believes that a little more than the pedal may possibly be represented by them.

Small nerves are given off from the cerebral ganglia (which ganglia are not large, but are very extended laterally, ganglion cells occurring along a nerve from which numerous branches go to the labial palps) to the mouth, lips, surrounding regions, and also to the digestive gland. The pedal ganglia are well developed and give off such a number of nerve branches into the foot that it is difficult to see how this organ can be regarded as rudimentary. Its tip is evidently highly sensitive.

There are no separate cerebro-pedal connectives of the usual type. This is unique, and since d'Hardiviller stated that he saw them, the present writer searched carefully through three series of sections for them. Any nerve of the thickness just visible in dissections would have been unmistakable in microscope sections. No such nerve was to be found. The result of the examination of the serial sections was, however, to find the two statocysts, both completely enclosed vesicles (no aperture to the exterior remaining). The nerves from the statocysts were exceedingly minute and only to be followed with great difficulty in the serial sections. Their course is naturally of great interest, for, if cerebro-pedal connectives had been present, these nerves would most likely have connected up with them. Instead of that, the statocyst nerves, after a rather long course, run into the cerebro-visceral connectives.

It is not possible to deny altogether the passage of nerve fibres from the cerebral to the pedal ganglia other than by the long path through the visceral ganglion, because extremely fine nerve branches, consisting of a few fibres only, are to be found with the microscope here and there in the visceral mass. It is unlikely that such a connection exists, but in any case typical cerebro-pedal connectives are absent.

The visceral ganglion (fig. 7) is, like that of *Pecten*, a most unusual structure to find in the Lamellibranchs, where the ganglia are as a rule rather simple masses. It might be termed the " brain " of the animal, for, from the point of view of both size, complexity and functions, it is the controlling centre.

Looking at the ganglion from the ventral surface one sees a prominent central lobe occupying the region of greatest thickness and two lateral crescentic lobes. (It will be seen that fundamentally this is the same as *Pecten maximus*, fig. 6, except that in the latter species the ganglion is asymmetrical, a feature which may be associated with the asymmetry of the Mollusc.)

Anteriorly (or perhaps one might better put it, orally) two large nerves leave the ganglion on each side. These nerves arise from the central mass and pass towards the hinge line on the surface of the adductor muscle just below the visceral mass. One of the nerves on each side is the pedo-visceral connective, the other is a nerve which splits later on, part going to the cerebral ganglion and the other part continuing as the Anterior Pallial nerve to enter the mantle margin near the hinge line.

In *Pecten* only one large nerve on each side is to be found, the cerebro-visceral connective (fig. 6, *Cer. Vis. Con.*). Between the cerebro-visceral connectives as they leave the visceral ganglion are two smaller ganglionic lobes, usually pigmented brown. The same region is present in *Spondylus*. The figure of the visceral ganglion of *Spondylus* given in the text is not, however, of such accuracy as that of *Pecten*. The present research was not carried out as a special investigation of the visceral ganglion, and the rarity of the material has made matters difficult. The *Spondylus* figure is the result of several very careful dissections. The *Pecten* figure resulted from a long study of serial sections and dissections. The fundamental features of the ganglion of *Spondylus* are, however, accurately depicted.

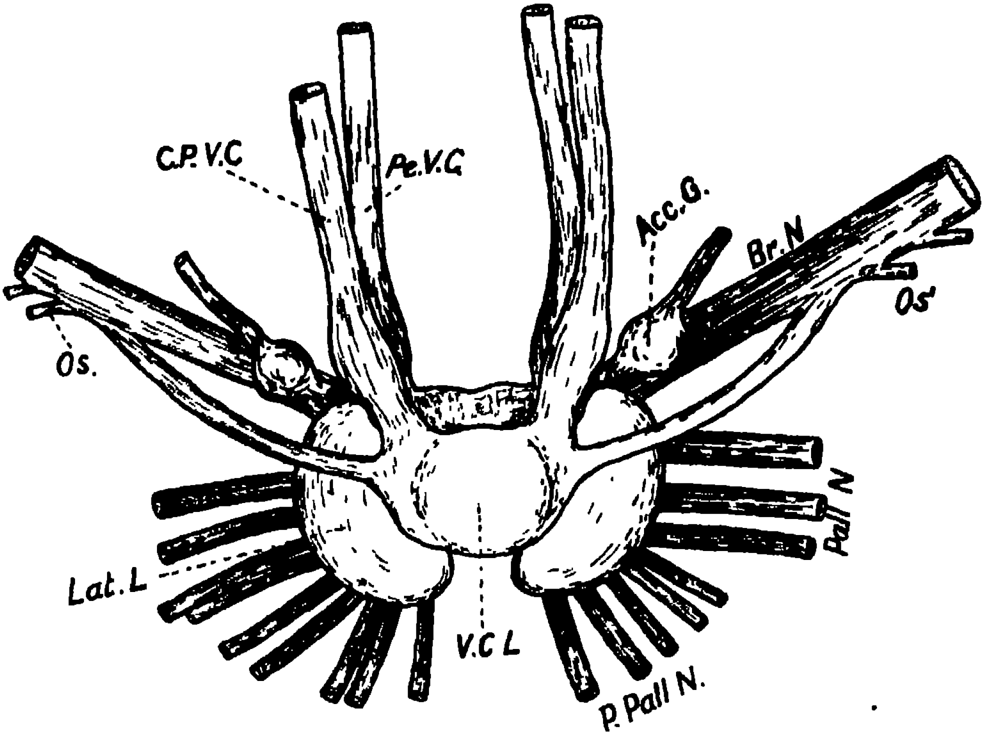

FIG. 7.—*Visceral Ganglion of Spondylus.* Acc. G., Accessory Ganglion; Br. N.; Branchial Nerve; C.P.V.C., Cerebropallial-visceral connective; Lat. L., Lateral Lobe; Os. Os'., Osphradial Nerves; Pall. N., Pallial Nerves; P. Pall. N., Posterior Pallial Nerves; Pe. V.C., Pedo-visceral connective; V.C.L., Ventro-central Lobe.

Two other small accessory ganglia remain to be mentioned: they are visible in both genera. In *Pecten* they are connected both with the visceral ganglion and also with the branchial nerves. Another branch leaves them for the viscera. In *Spondylus*, the small connection with the branchial nerve has not been observed.

The following nerves leave the visceral ganglion, apart from the cerebro-visceral connectives (and in *Spondylus* the pedo-visceral connectives) referred to above—the branchial nerves, the osphradial nerves and the pallial nerves.

The branchial nerves leave the ganglion in both *Pecten* and *Spondylus* near the point of entry of the cerebro-visceral connectives.

The osphradial nerves are peculiar and very characteristic. In both genera they arise from the face of the ganglion exposed to view and from what is apparently the central lobe. As a result each nerve crosses over the lateral lobe of its side. A little way from the ganglion it sends a branch into the branchial nerve and later still branches leave this nerve for the osphradium.

The pallial nerves of *Pecten* and *Spondylus* are particularly well represented, a fact to be correlated with the highly developed structure, including the eyes, tentacles and musculature, of the mantle edges. From the visceral ganglion there are posterior pallial nerves and lateral pallial nerves, and in *Spondylus* there is also an anterior pallial nerve, although at its commencement it is not distinguishable from the cerebro-visceral connective. All these pallial nerves are connected to a circular nerve which runs round in the mantle margins (see figs. 4 and 5, *Cir. pall.*).

Finally, nerves to the adductor muscle leave the visceral ganglion from the surface apposed to the muscle. They cannot be seen in the figures of the ganglion.

The internal structure of this complex ganglion has been described by Dakin (1910), and it has been shown that various tracts of fibres can be clearly made out in it. Reference should be made to the original paper for details. The structure of the lateral lobes of the visceral ganglion deserves mention, however, for these bodies appear to be characteristic of *Pecten* and *Spondylus*. The pallial nerves arise from their under surface and although some of the fibres of these nerves come from the more central parts of the ganglion and some actually from the cerebro-visceral connective, a considerable part comes from the lateral lobes. In fact, one may go further, and correlate the lateral lobes with the presence of the eyes. This might explain the difference between the size of the lobes of the right and left sides in both *Pecten maximus* and *P. jacobæus*, where the eyes are much more numerous on one mantle lobe than on the other.

A Possible Explanation of the Nervous System of Spondylus.

The question which now remains is the most interesting. Is it possible to explain the unusual features of the nervous system of *Spondylus* and to bring it in line with that of other Lamellibranchs? In my opinion this is not difficult, *if the conditions found in Pecten be used as the key.*

If the diagram of the *Pecten* nervous system (fig. 5) is studied, it will be noted that the cerebral ganglia are very close indeed to the pedal ganglia, and at the same time some distance away from their more usual position at the sides of the mouth. In consequence of this, the cerebral commissure is unusually long, and has to travel some distance to pass over the œsophagus. This feature has frequently been pointed out (Pelseneer and others) as rather characteristic of *Pecten*. Now in *Spondylus*, we must either explain the pedo-visceral connective as something new, which has suddenly appeared in this mollusc, which in so many other respects is a *Pecten*, or else we must look for the nerve in *Pecten* itself.

The clues are, to my mind, provided by the facts that in *Spondylus* (1) the cerebral ganglia are to be found in their normal position at the sides of the mouth and reduced in size; (2) a considerable part of the cerebro-visceral connective splits off just before reaching the cerebral ganglion and proceeds straight on, as an anterior pallial nerve to the mantle, near the hinge line. This latter fact points to the conclusion that in *Pecten* the pallial nerve from the cerebral ganglion does not really arise there at all; it only appears to do so because its fibres run from the visceral ganglion together with fibres which belong to the cerebral ganglion.

In *Spondylus*, however, for some reason or other, there is a tendency for nerves to split into their components. If we suppose that the cerebral ganglion itself has split (on each side), and that one part has remained with the pedal ganglion, whilst the other part has receded to the more normal position at the mouth, everything can be clearly explained. Each pedal ganglion would then have to be regarded as including part of the cerebral, whilst the cerebral ganglion proper would only be part of the ganglion bearing that name in *Pecten*.

It would then follow quite naturally that the "pedal" ganglion of *Spondylus* would be connected with the visceral, for the pedo-visceral connective would really be *part* of the cerebro-visceral connective of *Pecten* and all other Lamellibranchs. At first, this may seem to be rather difficult to understand, but the more one looks into the Lamellibranch system, and the more familiar one is with the nervous system of *Pecten*, the more plausible does it become.

This is not all however. In the Gastropoda there is a distinct ganglion, termed the pleural. This is usually supposed to be part of the *cerebral* of the Lamellibranchiata, and there is strong evidence for such an assumption. (In Pecten the cerebral ganglion on each side consists of two obvious lobes.) Now in some Gastropods, the pleural remains close to the cerebral ganglion. In others, it is situated closer to the pedal, and the pleuro-visceral connective is then almost a nerve from the pedal to the visceral ganglion. It is possible that the splitting of the cerebral (or cerebro-pleural) ganglion, which I believe has taken place in *Spondylus*, has left the pleural part of this nerve centre attached to the pedal ganglion. What we must call the pedo-visceral connective of *Spondylus* is then to be regarded as a strand of nerve fibres represented in *Pecten* and other Lamellibranchs by part of the cerebro-visceral connective.

One other feature of the *Spondylus* nervous system remains to be explained. There is no typical direct connection (cerebro-pedal connectives) between the cerebral ganglia at the lip-mouth angle and the ganglia of the foot. The only hypothesis which I can suggest is that such a direct connection may be quite unnecessary, if *part* of the cerebral (or the pleural) ganglion is really present in this form as part of the large foot ganglion. That is my theory. It supports most strongly the suggestions that the cerebro-visceral connective of *Pecten* has split, giving rise to the two connectives of *Spondylus*; and if such were not the case, *i.e.*, if the pedo-visceral connective of *Spondylus* were a new thing, a connection not even represented in the Mollusca, then one would also have to explain the entire absence of a cerebro-pedal connective. Thus, instead of this unique disposition of a Lamellibranch nervous system being a puzzle, it provides most interesting evidence for the evolution of the Spondylidæ.

Sense organs.

The eyes of *Spondylus* are discussed in the paper following. The other sense organs consist of the Osphradia, the Statocysts, the Abdominal Sense Organ and sensory cells and free nerve endings on gills, palps, foot and probably other parts of the surface of the body. The only organs of this group it is necessary to comment upon here are the statocysts. Although very small they are both present. This is quite in accordance with the fact that they are present in certain other fixed Lamellibranchs. It has been generally assumed that, although the Lamellibranch statocysts are often connected to the pedal ganglia, or to the cerebro-pedal connectives near the pedal ganglia, their nerve supply comes *really* from the cerebral ganglia. The state of affairs in *Spondylus* lends support to this view, for, had the nerve supply been from the pedal ganglia, one might have expected the statocyst nerves to run directly into them.

The Excretory and Reproductive Organs.

There is no reason to go into details regarding the structure of these two systems. They are not essentially very different morphologically from the corresponding organs of *Pecten*. Unfortunately, all the specimens except one were taken in non-breeding seasons and the gonads were thus in the reduced state. They lie in the visceral mass beneath the foot and, as stated previously, slightly invade the mantle in the region where they abut on the digestive gland. The animals are probably unisexual, but I should not like to be dogmatic upon this point. It is worthy of note that some species of *Pecten* are unisexual (*P. tenuicostatus* of America), whilst *P. maximus* and *P. opercularis*, our common English species, are hermaphrodite.

Conclusion.

The anatomy of *Spondylus* has been investigated in order to obtain evidence of the phylogeny of the genus, with a view to a discussion of the evolution of the eyes of *Pecten* and *Spondylus*. Jackson, in his classic work on the Phylogeny of the Pelecypoda, concluded that *Spondylus* had been evolved from *Pecten*. This theory was based entirely upon a study of the shell and upon palæontological discoveries—*Pecten* dates at least from Carboniferous times, *Spondylus* from the Triassic beds. The present work has not only shown that the anatomical evidence is overwhelmingly in support of this view, but it has indicated the line which has been followed, and shown how the more equivalve species of *Pecten*, like *P. opercularis*, represent the earlier and more primitive type, and that the direction which culminated in *Spondylus* was that which can be seen in the Pectinidæ, leading from *P. opercularis* through *P. jacobæus* and *P. maximus*.

The surprising discovery in relation to the nervous system shows that *Spondylus* must be regarded as the extreme of an evolutionary line, which probably commenced with the Aviculopectens of Palæozoic times.

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The Eyes of Pecten, Spondylus, Amussium and Allied Lamellibranchs, with a short Discussion on their Evolution.

By William John Dakin, D.Sc., Professor of Zoology in the University of Liverpool.

(Communicated by Prof. E. W. McBride, F R.S.—Received June 15, 1928.)

The eye of *Pecten* has excited an unusual amount of interest for many years, and many workers have described its structure. The present author devoted a considerable period to the study of its minute histology in 1908, and was able to make clear for the first time certain fundamental features in the structure of the retina. This work was confirmed in all essential points by Kupfer (1916).*

Several writers have commented upon the resemblances between the eyes of *Spondylus* and *Pecten*, although with the exception of Hickson's account of the structure of the *Spondylus* eye and a very brief reference by Hesse, there is no paper dealing especially with this genus. There is a general impression, too, that *Pecten* and *Spondylus* stand alone amongst Lamellibranchs in the possession of the most complicated type of eye, with two layers of sensory cells in the retina, and no mention appears in the literature of such genera as

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* Another reason the author had for returning to the study of the eye of the Pectinidæ was the appearance of a short paper by Roche, in the 'Journ. Roy. Micros. Soc.,' June, 1925. Roche, whose experience seems to have been rather limited, takes up his work on the basis that two points are left, on which Dakin and Kupfer are at variance. These are (1) the innervation of the distal sense cells, (2) the exact shape of the interstitial cells. It was a natural discovery for Kupfer, working on my data, to find that the innervation of the distal cells was by the distal processes and not by the side This is quite correct. It was recognised by myself long before I saw Kupfer's paper (after the War), and the correct innervation is shown in a text-figure published by me in 1921 (Inaug. Address, Univ. of Liverpool) four years before Roche's paper was published. There was, therefore, no disagreement between us on this.

In regard to the second point, the matter is more involved, although the exact shape of the interstitial cells is not a matter to worry over when so much more important research requires attention. Roche states that Kupfer considered these cells to be unbranched, whilst I regarded them as branched. Kupfer does figure them, rather diagrammatically, as unbranched, but on p. 190 he states—"Dakin has by the use of maceration methods been able to isolate these interstitial cells. We were not able to do this in a manner free from objection. *We have, however, no ground to doubt that when they are isolated they have the appearance figured by Dakin in fig.* 15*a*, *Plate* 7." There is, it will be seen, no variance here. Roche does not mention maceration methods in his paper.

Amussium, *Chlamys* and *Pedum*, which are, no doubt, very closely related to *Pecten*, although Ridewood placed *Amussium* in the family Mytilacea.

The development of the eye of *Pecten*, so far as it has been elucidated by Kupfer, throws little or no light upon the possible evolution of the eyes of the Pectinidæ. Comparison in the group thus becomes doubly interesting, and when I found myself in the fortunate position of obtaining living *Spondylus* at Naples, and small but valuable pieces of *Amussium* from the Natural History Museum, I realised there might be some advantage in examining carefully the eyes of *Spondylus* and those of other related genera.

Spondylus.

The eyes of *Spondylus gæderopus* were examined at Naples. Another species, *S. americana*, became available for comparison at a later date, when a single specimen collected by the United States Fishery Commission was kindly presented by the American Natural History Museum, Washington.

The eyes of *Spondylus* are situated in the same position on the mantle as the eyes of *Pecten*. In the species examined, they are on an average smaller than the eyes of species of *Pecten* of about the same dimensions. (There is, however, considerable variation in the size of the eyes in *Pecten* species.) The eye number is large compared with *Pecten*. Thus in *S. gæderopus* the numbers varied round 117 for the left valve and 58 for the right, whilst in the specimen of *S. americana* there were 92 on the left and 71 on the right valve. It is very interesting to note that the eye numbers for the right and left valves of *Spondylus* are related in the same way as those of *Pecten*.

Histological examination shows that the eyes closely resemble those of *Pecten* in structure. The resemblance, in fact, is much closer than has previously been suggested. The retina contains the same elements in the same positions (see accompanying diagram of structure of eye of *Pecten*). The smaller size of the eye, compared with, say, a large eye from *Pecten maximus*, is accounted for by the smaller number of cells present in the retina. (The same kind of variation in size due to differences in the number of retinal elements is found amongst the *Pecten* species, and even in one and the same specimen.)

Hesse states only one kind of difference between the eye of *Spondylus* and that of *Pecten*—the *septum* covering the retina of *Spondylus* is described as cellular. Nuclei are stated to be present, whilst neither nuclei nor cell boundaries are to be found in *Pecten*. I can find no support for this statement of Hesse. The structure of the septum of *Spondylus* does not appear

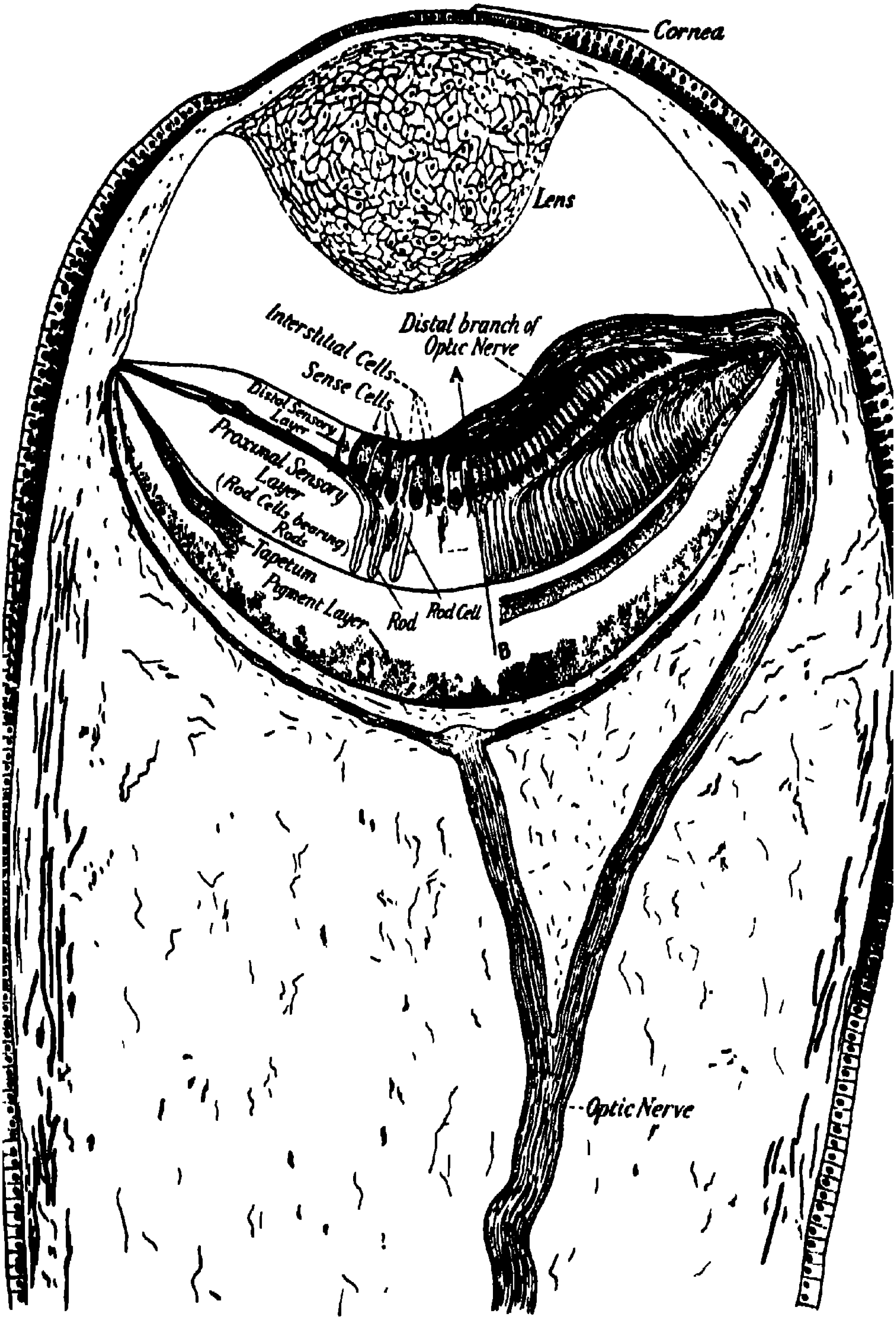

DIAGRAM of Structure of Eye of Pectinidæ.

The structure of the retina as it appears in sections is shown to the right of the line AB. The drawing to the left of the line AB illustrates on a larger scale the four components of the retina, Distal sense cells, Proximal sense cells Rod cells with Rods), Distal and Proximal Interstitial Non-Sensory Cells.

to be different from that of *Pecten*, and thus there is nothing to add to what has been said regarding this species (Dakin).

From the above (incorrect) observation, Hesse passes on to say that possibly *Spondylus* is a more primitive form, and that a cellular septum may have preceded the other type. There are no grounds now for such a suggestion, for all the evidence points to *Spondylus* having been derived from *Pecten* (see previous paper Dakin, 1928) and not to the reverse processes.

Chlamys.

This genus strikingly resembles *Pecten* in appearance, and is very closely related. The eyes are in all respects similar to those of *Pecten* and *Spondylus*. In the species examined there is one feature of note—the remarkable increase in the depth of the epithelial cells which form the cornea. In most species of *Pecten*, and also in *Spondylus*, the corneal cells are much smaller than those of the epithelium of the eye stalk. The peculiar cornea of *Chlamys* is not, however, confined to the genus, for the author can remember meeting the same type, although not so exaggerated, in an unknown species of *Pecten* examined at Naples many years ago.

Amussium.

This rather rare bivalve presented features of particular interest, because Ridewood removed the genus from the family Pectinidæ altogether, after his examination of the gill structure of the Lamellibranchs, and relegated it to a different sub-order, that of the Mytilacea. Ridewood's work on the lamellibranch gills was thorough and his classification based upon the gills has proved of considerable value. It is very questionable, however, if it is fair to classify any animal group entirely on the basis of the structure of some single organ. In the case of *Amussium* there can be little doubt that the method adopted led Ridewood astray, and the writer would suggest that both on the ground of general anatomy, as well as on the evidence from a study of the eye, *Amussium* be brought back again to the Pectinidæ.

The eyes of *Amussium* are again so very like those of *Pecten* that it is doubtful whether an expert could distinguish sections of one from sections of *Pecten* species. It was not possible to be absolutely certain about the most minute points, because the only material available (kindly presented by the Natural History Museum) had been in spirit for many years. The preservation of the general structure was, however, excellent, and so close is the resemblance that it is improbable that any differences in minute structure exist greater than those found amongst species of *Pecten.*

Pedum.

A small part of the mantle of *Pedum* was examined, but the specimen was in extremely bad condition, and it was quite impossible to make out the histology of the eyes, which appear to be present in the same position as those in *Pecten.*

Summary of Facts regarding Eye Structure.

1. The conclusion reached by the examination of the genera referred to above is that several genera of Lamellibranchs have eyes with the now well-known complex structure of those of *Pecten.* The genera include *Spondylus, Amussium, Chlamys* and most probably *Pedum.*

2. Consequent upon a study of these and other lamellibranch visual organs, it may be affirmed that the peculiar structure so characteristic of *Pecten* eyes (the cellular lens, the double retina with two distinct layers of sense cells which are characteristically arranged) is confined to the family Pectinidæ. There are no other genera outside this group with eyes of the *Pecten* type, the nearest approach being those of *Cardium.*

3. Within the family Pectinidæ the eyes are so uniform that it is impossible to indicate any differences which could be regarded as characteristic of the different genera. Even the eyes of *Amussium* (which must be brought back to the family Pectinidæ on the grounds of the close resemblance in all the general anatomical features) agree in detail with those of *Pecten* and *Spondylus.*

4. No evidence regarding the evolution of the *Pecten* type of visual organ can be obtained from a comparative study of these or other Lamellibranchiata.

The Evolution of the Eyes of the Pectinidæ.

Charles Darwin was very considerably impressed by the difficulties facing him in an attempt to explain the evolution of the vertebrate eye on the basis of Natural Selection. He devoted a special statement to the eye in 'The Origin of Species,'* and from this the following extract is taken:—

"When we reflect on these facts, here given much too briefly, with respect to the wide; diversified and graduated range of structure in the eyes of the lower animals; and when we bear in mind how small the number of all living things must be in comparison with those which have become extinct, the difficulty ceases to be very great in believing that natural selection may have converted the simple apparatus of an optic nerve . . . . into an optical instrument as perfect as is possessed by any member of the Articulate Class.

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* 'Origin of Species,' 6th ed., page 135.

"He who will go thus far ought not to hesitate to go one step farther, if he finds on finishing this volume that large bodies of facts, otherwise inexplicable, can be explained by the theory of modification through natural selection; he ought to admit that a structure even as perfect as an eagle's eye might thus be formed, although in this case he does not know the transitional states."

This is an optimistic view of the problem rather than evidence; it is a view to which I find it very difficult to subscribe in so far as the eyes of the Pectinidæ are concerned. Indeed, after a careful comparative study of the visual organs of the invertebrates, one finds greater difficulty in accepting the principle of natural selection as the dominant factor in their origin than is the case with any other of their morphological features.

In the bivalve molluscs we meet with many types of eyes, but there is only one known up to date (in the genus *Cardium*) which might be said to present the same type of complexity exhibited by the Pectinidæ. Within the Pectinidæ, as we have seen, there is a remarkable uniformity. The *Pecten* type of eye occurs only in a group of lamellibranch genera which are extremely closely related (but for the shells it is doubtful whether their differences could really be said to be of generic rank).

Now it is very difficult to conceive of a complex structure, complex as these eyes, as being the final result of a sifting by natural selection of a large number of "chance" variations, stress being laid on external factors. Indeed, there is grave doubt as to whether the presence of any variations that might lead to such organs could have any "survival value." Are there any indications that ancestors of the Pectinidæ, or present members of the group, with variations in the negative direction would be unfavourably biassed thereby? An attempt may be made to answer this question by a study of the habits of lamellibranchs possessing highly developed visual organs and by making comparisons with less well-equipped forms.

There is a remarkable diversity of structure in the eyes of the Invertebrata, even in one and the same phylum, and this diversity is particularly well expressed in the still smaller group, the class Lamellibranchiata, where other organs are so well graded that several schemes of classification have been adopted and given up on this account. The diversity is clearly due to independent origin. We know that natural selection does not "produce" anything, but one might have expected that in a group like the Lamellibranchiata, if natural selection were indeed a powerful factor, there would have been closer agreement in the structure of the visual organs. On the contrary, we must regard each type as an independent expression of the working of the factors involved in evolution, whatever these may be.

Let us regard the eye of the Pectinidæ from the point of view of function. We cannot express our opinion upon the delicacy of vision in our example without making experiments. A study of structure alone leads to a most confusing position at the outset. Von Uexküll showed how in the sea-urchin a whole collection of organs was co-ordinated and bound up by a common nerve net, in contrast to the conditions in higher animals, where fewer sense organs and a differential nervous system are more efficiently utilised. The presence of a large number of eyes in *Pecten* species, together with what is, on the whole, a simple nerve connection, is a similar case and speaks for little specialisation in function. In complete contrast to this the remarkable complexity in eye structure (always commented upon in the literature) points to efficient and specialised function. This clash itself prepares one for doubt as to the efficiency of the *Pecten* eye.

Notwithstanding this beautiful and complex structure, I regard a belief in high efficiency to be an unwarranted deduction. I have kept large numbers of *Pecten* in aquarium tanks and experimented on their reactions to shadows thrown on the eyes as well as to sudden increases in light A moving shadow thrown on a number of eyes, frequently but not always results in a brisk reaction on the part of the animal. *Pecten* does not appear to show much preference for the illuminated or non-illuminated parts of large tanks. The reaction to light does not appear to be more marked than is the case with other Lamellibranchs not nearly so well provided with visual organs.

Von Uexkull, after conducting several experiments, came to the conclusion that *Pecten* reacted to the vision of a moving starfish, and associated this with the fact that the starfish is a natural enemy of the bivalve. A metallic model of a starfish was stated to produce a response, and also the shadow of the open hand when moved. The response is stated to be twofold, the first step being the extension of the long tentacles of the mantle edge; this is followed by flight if the shadow is that of a starfish.

My earlier experiments (described in 'Mitt. Zool. Stat. Neapel,' 1910–1913) caused me to deny the existence of any difference between the response of *Pecten* to the shadow of an object shaped like a starfish and the response to the shadow of a moving object of any other shape. A later opinion of Von Uexküll is expressed in his last edition of 'Umwelt und Innenwelt der Tiere.'

I notice that his views are now more in agreement with mine, although he still regards a moving starfish as having a particularly potent shadow. He states that "Die Form und Farbe des Gegenstandes auf die Muschel, d.h., das Bild auf der Netzhaut, wird nicht zur Erregung benutzt. . . . Eine Bewegung von ganz bestimmter Geschwindigkeit . . . wird zum Erregung auslosenden Reiz." I have shown (Dakin 1910–13) that to put a starfish actually amongst a number of *Pecten* in an aquarium is a very certain way of producing a lively commotion, but the same unrest can also be achieved by adding starfish *pulp*. The reaction is therefore more likely brought about by chemotactic sense organs rather than by way of the eyes. In any case the response of a *Pecten* to a shadow such as that thrown by Von Uexküll's model is only another example of a type of reaction met with not infrequently amongst invertebrates, and does not imply the perception of an image.

Most bivalves can only push themselves laboriously over the sand or mud of the sea bottom, and a few live attached to rocks or other firm objects, but *Pecten* can swim. Its swimming is so fantastic and unexpected that it invariably arouses the excitement of an onlooker when seen for the first time. Nothing could be more natural than to explain away the peculiarities of the visual organs of *Pecten* by relating them to this power of locomotion. But against this we have other lamellibranch examples. Thus *Arca*, a slow or non-moving bivalve, has very well-differentiated eyes. The species of the genus *Lima*, which are more beautiful and active swimmers than *Pecten*, exhibit remarkable differences. *Lima excavata* and *L. squamosa* have eyes—only poorly developed compared with Pectinidæ (they are little pits without a lens), whilst, according to Hesse, *L. inflata* and *L. hians* have no eyes at all.

Another surprise is *Spondylus*, for the genus does not swim, but is attached to rocks. It must be admitted, however, that the sedentary habits of *Spondylus* cannot altogether be brought forward as demonstrating that unusual activity and eye development cannot be correlated. The previous paper has shown clearly, I think, that *Spondylus* had swimming ancestors. *Spondylus* has actually inherited its eyes and its mantle edge from *Pecten*. It *is* an interesting fact, however, that such minute histological features as those of the *Spondylus* retina should have remained constant through ages, *i.e.*, from Triassic times (a period which has seen many tremendous evolutionary developments), although the habits of the animal have been unlike those of the ancestor from which the eyes were inherited. If, however, we regard the eyes as of rather unnecessary complexity for the duties they are called upon to perform in the *Pectens*, it becomes more easy to explain why they remain in *Spondylus*. Probably they are just as useful in the latter creature as in the lethargic *Pecten maximus*, which very likely only "swims" for very short distances and infrequently.

The contention suggested above is that the size and complexity of the eyes in Pectinidæ is not to be explained by natural selection. The difficulty of correlating the complexity of eye structure with function was evidently felt many years ago by Patten, when he formulated the theory which raised a storm of criticism. He suggested that the structures termed eyes were not really visual organs, but organs for the reception of light energy, to be utilised for metabolic purposes; he called them Heliophags. Probably such a theory would be still unacceptable for any such organs in the animal kingdom, although, in view of the discoveries of modern times, I for one would not like to deny the possibility of animals utilising radiant energy (apart, of course, from those containing chlorophyll).

Swimming, in the case of the scallop, is accomplished by movements which are not so simple as they seem at first sight. The water is trapped by the muscular mantle edge and compelled to leave the shell dorsally near the hinge line. The animal moves with the ventral margins of the shell foremost, in a series of jerks, just as if taking a succession of bites out of the water. These movements will require organs of orientation for their proper control. The water could be easily expelled in a manner so that the first shell "claps" were unsuited to raise the creature from the sea bottom. Statocysts are present, it is true, but it is conceivable that the eyes might play a part in orientation rather similar to that of the ocelli in Medusæ, where no one would look for image formation or image recognition.

In both the Arthropoda and the Fishes, two very different groups of animals, the influence of the deep sea is expressed in the same way—some forms have larger eyes than usual, some show eyes in stages of degeneration. It is difficult to understand how selection of small variations in two diametrically opposite directions could occur at the same time and under the same conditions. The evidences of degeneration, and particularly the work of Kammerer on *Proteus*, favour Lamarckian explanations for the reduction of the eyes in cave forms and other similar examples. Whether it is rational to extend the application of the same principles to the tendency to develop the size and complexity of eyes, is an important question.

It is characteristic of the distribution of visual organs that they appear on the part of the animal most directly opposed to the light rays. It would not be speculative therefore to assume that the direct effect of radiant energy had at least called forth the beginnings of such sense organs. It is perhaps unorthodox to say that Lamarckian principles may have played some rôle in moulding their further development. But it may clearly be argued that if a lens may regenerate in the Amphibia from the edge of the iris, after destruction of the normal lens—a mode of origin which is so abnormal that it does not occur in ontogeny—there is no reason why changes in external stimuli and modification in habits should not also call forth directly adaptive changes in structure in sense organs of the type we are considering. That, however, is a possibility I cannot follow here.

Whatever may have been the *origin* of the eyes of the *Pecten* group, the point I wish to stress is that their subsequent development in size and complexity was the consequence of internal factors—was, in fact, orthogenetic. I do not hold that utility explains their evolution. I do not, of course, necessarily deny the action of selection altogether in the phylogenetic vicissitudes which have led to Molluscs as a group to-day, but feel that in the case of the Pectinidæ there is no evidence of the elimination of types with less complex eyes as unfit—that, in view of the diverse conditions existing in the Lamellibranchs, there is no evidence that a reduction in the efficiency of the eyes of *Pecten* would lead to unfitness—and that in any case, the complexity of structure has far outstripped the needs of the animal and the efficiency of the eyes themselves.

We cannot escape from the conviction that in one particular series of bivalves, all intimately related genetically, a distinct type of visual organ arose, independent of other visual organs (except possibly of *Cardium*-like ancestors), and that apart from adaptation, and apart from utility or advantageousness (as Kyle puts it), it attained a certain extraordinary complexity.

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Investigations of the Cell-wall Substances of Plants, with special reference to the Chemical Changes taking place during Lignification.

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As the result of various investigations, to which reference will be given in the text of this communication, it is possible to divide the substances which accompany cellulose in the cell-walls of plants among the following groups :—

(i) The lignins. (ii) The hemicelluloses. (iii) The pectins.

It cannot yet be claimed that any one of the products which can be assigned to these groups has been isolated in the form of a definite chemical entity (with the exception, perhaps, of some products in group iii). There are, however, certain characteristics common to each group, to which brief reference must be made.

The lignins appear to be products of high molecular weight, which can be extracted by various somewhat drastic methods from woody tissues. The products obtained by different methods vary in their properties, but they appear to give definite colour reactions with a number of reagents.

The hemicelluloses include those substances which were originally described by Schulze, to whom this name is due, which cannot be extracted from the tissues by water, but which are readily dissolved by solutions of caustic alkalis of about 4 per cent. strength. The pectins cannot be extracted with alkalis or water, but are readily obtained by warming the tissues with dilute (0·5 per cent.) solutions of ammonium oxalate, and other salts, which yield, by double decomposition, calcium compounds insoluble in water. The pectins are characterised by their ability to form gels under various conditions.

Lignified and non-lignified tissues differ from one another in many respects. The former contain, in addition to cellulose, lignins and hemicelluloses in appreciable quantities, and only small traces of pectins.* In the non-lignified tissue, on the other hand, lignins are absent; hemicelluloses are present only in small quantities, whereas the pectins are present in relatively large amounts.† During lignification, therefore, the pectins disappear, to be replaced by hemicelluloses and lignins.

In the course of the investigations on these constituents it was found that the hemicelluloses obtained from non-lignified tissues varied in their properties according to whether they had been extracted before or after the separation of the pectins. Furthermore, when pectinogen, the name given to the soluble product extracted by ammonium oxalate solutions from the tissues, is treated with cold lime-water it yields, with elimination of methoxyl groups, calcium pectate, the calcium salt of pectic acid, which is insoluble, together with a small amount of hemicellulose.‡

The variations in the properties of the hemicelluloses, and the relationship of these substances to pectins, are difficult to explain, unless the hemicelluloses undergo a change during their separation from the tissues, due to the action of the reagents employed for their extraction. It was decided, therefore, to undertake a systematic investigation of the action of these reagents on the different preparations obtained. This communication describes the results of the action of sodium hydroxide in varying strengths on pectic acid, the best defined of the products hitherto obtained. They indicate that pectic acid, under the influence of the alkali, readily undergoes decarboxylation, yielding amongst other products, a substance similar in all its properties to the hemicelluloses obtained in relatively large amount from lignified tissues. There is evidence, therefore, that hemicelluloses found in lignified tissues are derived directly from pectins by simple decarboxylation, a change which can be brought about *in vitro* by weak alkalis at relatively low temperatures.

The quantity of hemicelluloses obtained by this method amounts only to about 12 per cent. to 20 per cent. of the pectin decomposed; other products are obtained in addition, and it is tempting to suggest that these may be related to the lignins. Up to the present, however, no evidence has been obtained to support this view. The substances yielded by decarboxylation, in addition to hemicellulose, are of relatively simple character, and pass through a parchment dialysing membrane. If the lignins (which, as already mentioned, are substances of high molecular weight*) are derived from the pectins, two possibilities exist: either the action of alkalis, as described below, produces too great a degradation of the pectins, or the lignins are produced from the simple degradation products by a subsequent synthetical process, which takes place in the plant. It is of interest to note that the lignins contain no uronic acid groups.

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* *Cf.* M. H. O'Dwyer, 'Biochem. J.,' vol. 17, p. 501 (1923); vol. 19, p. 694 (1925); and vol. 20, p. 656 (1926).

† Clayson, Norris and Schryver, 'Biochem. J.,' vol. 15, p. 643 (1921).

‡ Norris and Schryver, 'Biochem J.,' vol. 19, p. 676 (1925).

Characterisation of the Products.

The hemicelluloses hitherto investigated in this laboratory, and the pectins, are all derivatives of sugar acids, such as glycuronic and galacturonic acids, for which the convenient name of " uronic acids " has been suggested by Prof. Ling. Miss O'Dwyer (*loc. cit.*) succeeded in isolating both the above-mentioned acids from two different hemicelluloses isolated from beech-wood. The pectin and hemicellulose molecules appear to be built up by the conjugation of such acids with sugars. They both may be regarded then as belonging to a definite class of chemical substances, for which the name " polyuronides " is suggested.

Owing to the presence of these uronic acid groupings they yield, on treatment with hot 12 per cent. hydrochloric acid, carbon dioxide and furfural. The products described below have been characterised, therefore, by the amounts of carbon dioxide and furfural they yield by this treatment. The former was determined by the method suggested by Nanji, Paton and Ling,† and the latter by the well-known method of Wheeler and Tollens.

Preparation of Pectic Acid.

Two sources of material were employed, viz., (i) pectinogen of onions, (ii) pectinogen from citrus. The former was prepared by the methods described by Clayson, Norris and Schryver (1921), and Norris and Schryver (1925) (*loc. cit.*). The latter was a commercial product made in the United States of America. Its actual method of preparation is unknown. The pectic acid was prepared in the following manner :—

To a 1 per cent. solution of the pectinogen, in water, an equal volume of lime-water was added, with stirring, and the mixture allowed to stand for 24 hours.

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* The molecular weight of lignin appears to be about the order of 800. *Cf.* Fuchs, 'Die Chemie des Lignins,' Berlin, p. 424 (1926).

† 'J. Soc. Chem. Ind.,' vol. 44, p. 253 T (1925).

The gel of calcium pectate was then filtered off on fine muslin, and well washed with cold water. After being pressed as free as possible from liquid, the gel was dissolved in warm ammonium oxalate solution. The precipitate of finely divided calcium oxalate was removed by filtering the liquid through a thick pad of paper pulp. Pure concentrated hydrochloric acid was added to the filtered solution of ammonium pectate, and after standing for several hours the gel of pectic acid which had separated was filtered off on muslin, well washed with dilute hydrochloric acid till the washings gave no precipitate with ammonia, and then with cold distilled water till the washings were free from chloride. The gel was then dried by graded strengths of alcohol, and finally over sulphuric acid in a vacuum desiccator, whereby a yield of 60 per cent. of the acid was obtained, reckoned on the original pectinogen.

The Action of Alkalis of Varying Concentrations on Pectic Acid.

In the following experiments about 0·2 gramme of the acid were allowed to stand for varying times and at different temperatures with 75 c.c. of alkali. Hydrochloric acid was then added till the concentration was 12·5 per cent. HCl, and air was passed through the cold solution to remove the carbon dioxide produced from the carbonate formed. The mixture was then heated, and the carbon dioxide evolved was trapped in a series of wash-bottles containing barium hydroxide. The amount evolved was estimated by titrations by the method of Nanji, Paton and Ling (*loc. cit.*).

Preliminary experiments indicated that 12·5 per cent. hydrochloric acid in the cold does not attack uronic acid complexes. The action of alkali causes decarboxylation, so that less carbon dioxide is evolved after treatment with strong hot acid. This is expressed in the following tables in the form of percentage diminution of the total evolved from the pectic acid before treatment with alkalis. The results are shown graphically in the accompanying curves.

Table I.—Decomposition of Onion Pectic Acid by Alkalis.

Strength of Alkali.	Temperature.	Time of action.	Percentage loss of uronic anhydride.	
			A.	B.
Normal (4 per cent.)	18° to 19° C.	2 days	2·15	2·01
,, (4 per cent.)	18° to 19° C.	4 ,,	4·0	4·0
,, (4 per cent.)	18° to 19° C.	7 ,,	7·0	6·75
,, (4 per cent.)	18° to 19° C.	11 ,,	11·2	11·1
,, (4 per cent.)	37° to 38° C.	2 ,,	10·4	10·0
,, (4 per cent.)	37° to 38° C.	10 ,,	18·4	19·3
,, (4 per cent.)	37° to 38° C.	14 ,,	18·7	19·7
,, (4 per cent.)	50° C.	2 ,,	30·7	30·4
,, (4 per cent.)	50° C.	9 ,,	41·0	42·4
,, (4 per cent.)	50° C.	35 ,,	69·3	68·7
,, (4 per cent.)	* 100° C.	4 hours	85·0	84·0
1 per cent.	35° to 36° C.	2 days	13·5	14·8
1 per cent.	35° to 36° C.	4 ,,	19·7	19·1
1 per cent.	35° to 36° C.	10 ,,	22·0	20·7
0·5 per cent.	35° to 36° C.	2 ,,	19·8	20·3
0·5 per cent.	35° to 36° C.	4 ,,	26·9	25·5
0·5 per cent.	35° to 36° C.	10 ,,	30·0	39·0
0·5 per cent.	* 100° C.	30 mins.	52·4	52·8
0·5 per cent.	* 100° C.	1 hour	67·0	—
0·5 per cent.	* 100° C.	4 hours	83·6	83·6

(Series A and B are duplicate experiments.)

* By "refluxing" with alkali.

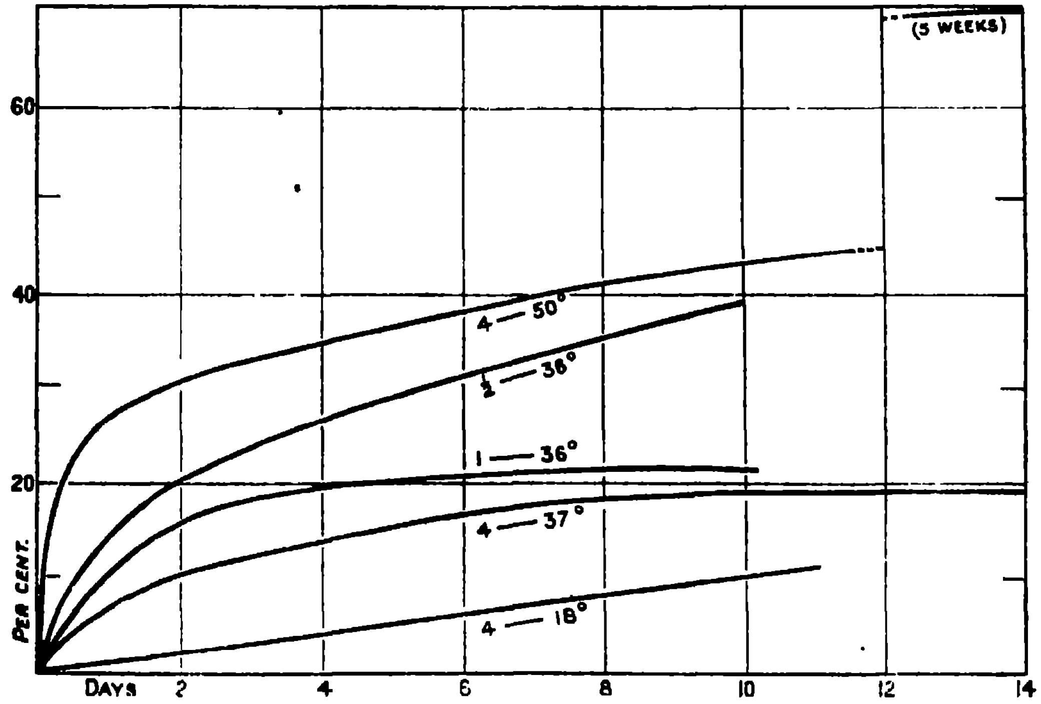

FIG. 1.—Decarboxylation of Onion Pectic Acid by Caustic Soda of percentage strengths and at temperatures shown.

Table II.—Decomposition of Citrus Pectic Acid by Alkalis.

Strength of Alkali.	Temperature.	Time of action.	Percentage loss of uronic anhydride :	
			A.	B.
0·5 per cent.	* 100° C.	30 min.	33·1	33·7
0·5 per cent.	* 100° C.	4 hours	80·0	80·0

(Series A and B are duplicate experiments.)
* By "refluxing" with alkali.

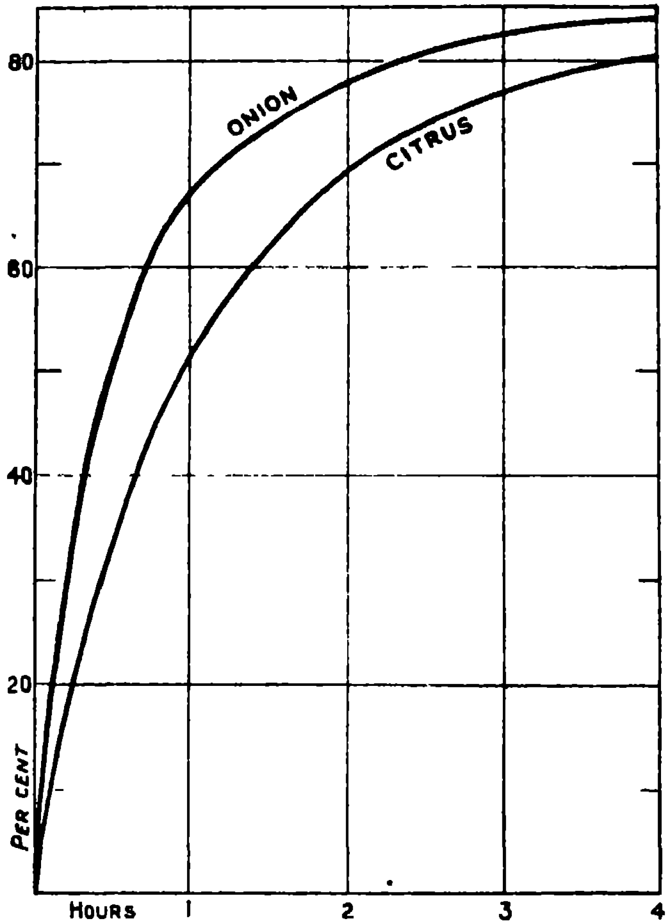

FIG. 2.—Decarboxylation of Citrus and Onion Pectic Acids with 1/8 N. Caustic Soda at 100° C.

Action of Heat on Sodium Pectate.

The above experiments indicate that weak (0·5 per cent.) alkaline solutions are as efficient (if not more so) in producing decarboxylation as the stronger solutions. It was therefore of interest to ascertain whether sodium pectate undergoes decarboxylation on boiling with water, without the addition of excess of alkali. A sample of the sodium salt was, therefore, prepared by dissolving pectic acid in a slight excess of 1 per cent. sodium hydroxide solution; to this solution, about twice the volume of alcohol was added, whereby the sodium pectate was precipitated. This, after filtration, was washed with 95 per cent. alcohol till the washings were free from alkali, and finally allowed to stand in absolute alcohol, and dried in a desiccator. 0·2 grammes of the salt were then dissolved in 75 c.c. of water, and the uronic acid content of this solution determined. After heating for four hours no diminution on uronic acid content was observed. Free alkali is therefore necessary to produce the decarboxylation of the sodium salt.

An experiment was carried out in which 0·1 per cent. sodium hydroxide was used. This was found to produce, at 100° C., slow decarboxylation, which amounted to about 26 per cent., as compared with 80 per cent produced by 0·5 per cent. sodium hydroxide in the same time. In all subsequent experiments decarboxylation of pectic acid was carried out with 0·5 per cent. sodium hydroxide at 100° C.

Preparation of Hemicellulose from Pectic Acid by the action of Sodium Hydroxide.

(*a*) From citrus pectic acid. 6·04 grammes of the acid were dissolved in 2265 c.c. of 0·5 per cent. sodium hydroxide solution, and the mixture was then boiled under a reflux condenser for four hours. After neutralisation with hydrochloric acid, the solution was evaporated under reduced pressure to 230 c.c. The solid which separated was filtered off and dried. The powder thus obtained weighed 0·6 gramme. The dried material had a uronic anhydride content of 64·5 per cent., which diminished to 33·2 per cent. after heating. It had all the properties of unchanged sodium pectate (uronic anhydride content 66·5 per cent.). To the filtrate from this substance was added an equal bulk of 95 per cent. alcohol. After standing overnight a precipitate was obtained, which, after washing and drying with graded strengths of alcohol and *in vacuo*, in the usual manner, weighed 0·8 gramme. It formed a greyish powder when dry, which dissolved readily in water to give a neutral solution. The percentage of uronic anhydride it contained was 37·3, which did not diminish at all after heating with N/8 sodium hydroxide solution for four hours at 100° C. Its solution did not reduce Fehling's solution, but it readily yielded reducing substances after boiling with 4 per cent. sulphuric acid, as the following figures indicate:—

Table III.

Time of hydrolysis (hours).	Mgms. reducing substance, calculated as glucose, per gramme substance.
0	0
2	270
4	313
6	355
13	364

The furfural content was found to be 17·4 per cent. All the results of analyses are calculated for the ash-free substance. This product has all the characteristics of a hemicellulose; a more detailed comparison is made below.

The substances in the filtrate from the hemicelluloses were submitted to further examination, of which a description is given in a later section.

(*b*) From onion pectic acid. The pectic acid (6 grammes) from onions was heated with 0·5 per cent. sodium hydroxide solution in precisely the same way as that from citrus, and the same procedure was adopted for the isolation of the products. On concentration, after neutralisation, only 0·2 gramme of the unchanged pectate was isolated; on the addition of alcohol to the filtrate a precipitate, weighing 1·2 grammes when dry, was obtained. This had very similar properties to the hemicelluloses isolated from the citrus pectic acid. Its uronic anhydride content was 21·5 per cent., and did not diminish after heating for four hours with 4 per cent. sodium hydroxide solution. It yielded 8·43 per cent. of furfural (all analyses calculated for the ash-free product). It did not reduce Fehling's solution until after boiling with 4 per cent. sulphuric acid solution, as the following figures indicate:—

Table IV.

Time of hydrolysis (hours).	Mgms. reducing substance, calculated as glucose per gramme substance hydrolysed.
0	0
2	712
4	820
6	867

Several other similar experiments have been carried out which gave the same results. Mr. H. S. Sharma has found that the pectic acid from turnips also readily undergoes decarboxylation on treatment with caustic alkalis.

A Comparison of the Hemicelluloses obtained by the Decarboxylation of Pectic Acids, with those isolated directly from Lignified and Unlignified Tissues.

It has been shown above that the pectic acids with high uronic anhydride content readily undergo decarboxylation to yield hemicelluloses, which also contain uronic acid groups, though these are present in much smaller quantity than in the pectic acids from which they were obtained. A marked characteristic of these hemicelluloses is that although they contain these uronic groupings, they do not undergo further decarboxylation on prolonged treatment with boiling 0·5 per cent. sodium hydroxide solution. It was of interest, therefore, to compare them with the hemicelluloses isolated directly from plant tissues.

The hemicelluloses were prepared from the cell-wall substance of turnips by the method described by Clayson, Norris and Schryver (*loc. cit.*). These were obtained in two fractions, designated A and B, by the following method. The alkaline extract was acidified by addition of a small excess of glacial acetic acid. A precipitate separated, from a somewhat turbid supernatant fluid, the greater part of which was syphoned off after standing overnight. The precipitate was separated from the remainder of the mother-liquor by centrifuging. It was then re-dissolved in a small amount of 4 per cent. sodium hydroxide solution, from which it was re-precipitated, after clarification by kieselguhr (2 grammes per 100 c.c.) by the addition of acetic acid and alcohol, the latter being added in bulk equal to that of the aqueous fluid. The precipitate was dried in the usual manner, and constitutes the fraction designated A. The mother-liquor from this fraction was then concentrated to one-fifth of its original bulk, after clarification by kieselguhr (2 grammes per 100 c.c.), and twice the volume of alcohol was then added. In this way hemicellulose B was precipitated, which was washed and dried in the usual manner.

Two hemicelluloses were isolated from beech-wood, precisely in the manner described by Miss O'Dwyer (*loc. cit.*). The uronic anhydride content in these hemicelluloses was determined, both before and after heating, with 0·5 per cent. sodium hydroxide solution, and the following results were obtained :—

Table V.

Uronic anhydride content.	Turnip.		Beech-wood.	
Per cent.	A.	B.	A.	A + B (mixture)
Before treatment with alkali	15·4	10·7 9·7	11·5 11·1	18·8 19·2
After " " "	13·3	6·8 6·95	10·4 11·0	18·5

It will be seen that the hemicelluloses from non-lignified tissue undergo a slight amount of decarboxylation on treatment with alkali, whereas those from lignified tissue remain intact. There is therefore a great similarity between the hemicellulose of lignified tissue and those obtained by the action of alkali on pectic acids. The fact may also be recalled that all readily yield reducing sugars on hydrolysis with weak acids.

The Products, other than Hemicellulose, produced by the Action of Alkalis on Pectic Acid.

Up to the present time, the products other than hemicelluloses obtained by the action of alkalis on pectic acids have not been identified. The mother-liquors from which the hemicelluloses were precipitated were neutralised with hydrochloric acid, and evaporated to dryness, *in vacuo*. Brownish masses, containing large amounts of sodium chloride, were obtained. The organic matter was insoluble in absolute alcohol, but soluble in alcohol containing a small amount of water. It was free from uronic acid groups, and did not contain any reducing sugars, and only small amounts of substances which reduce Fehling's solution were obtained after hydrolysis with 4 per cent. sulphuric acid solution. The substances it contained were mostly of simple character, and more than 95 per cent. passed a parchment dialysing membrane. It is possible that they are products of decomposition of sugars, produced by the action of hot alkaline solutions, as it might be expected that such substances are set free from pectic acids by the action of alkali.

It was found that arabinose, on heating for half an hour with 0·5 per cent. sodium hydroxide solution, lost 90 per cent. of its reducing power, and gave rise to products not unlike those just described. Attempts were made to separate the organic matter from sodium chloride. In one experiment the hydrolysis was carried out with the use of N/8 lithium hydroxide solution, and separation of the greater part of the lithium, after its action, as phosphate. Although the normal decarboxylation took place, it was still found impossible to obtain the organic matter free from contamination by inorganic salts.

Although the products, other than hemicelluloses, obtained by the action of alkalis on pectic acids are probably decomposition products of simpler sugars, it was nevertheless regarded as desirable to ascertain whether any relationship could be established between them and the lignins—to find, *e.g.*, whether they gave the same colour reactions. For this purpose a sample of lignin was made from pine wood, by extraction of the sawdust with alcoholic hydrochloric acid solution, by the method of Friedrich and Diwald.*

The lignin thus isolated was found to be free from uronic acid groups, and gave, both before and after heating for some hours with 0·5 per cent. sodium hydroxide solution, the typical lignin colour reactions with the following reagents: aniline sulphate, phloroglucinol, carbazole, benzenediazonium chloride, Millon's reagent, and Tollens' silver reagent. None of these reactions were given by the products of undetermined nature obtained by the action of alkalis on pectins.

If there is any relationship between the pectins and the lignins, then the change takes place in some way other than that brought about by the action of alkalis, or, what is perhaps more probable, the lignin is produced in the tissue by synthesis of some of the degradation products.

A Comparison of the Pectic Acids from Citrus and Onions.

The pectic acid from onions and a number of other plant materials has already been prepared by Clayson, Norris and Schryver (*loc. cit.*). The acids obtained from the different sources, with the exception of that from citrus fruits, appeared to be identical. It is of interest to note that the pectic acid obtained from the citrus by the method described in this paper differs from the acid obtained from onions, not only in its own uronic anhydride content, but also in the uronic anhydride content of the hemicellulose obtained from it by the action of alkalis. It would appear as if the citrus pectic acid contained a larger number of uronic groups in its molecule than do most of the other pectins. It is not proposed to discuss this matter in detail here, but the following table is appended to indicate the differences between the two pectic acids employed in these researches.

Table VI.

—	Citrus.	Onion.
	Per cent	Per cent
Uronic anhydride content of pectic acid	89·0, 88·2	69·7, 70·3
Furfural content of pectic acid	23·15, 23·0	18·2, 18·1
Uronic anhydride content of derived hemicellulose	37·3, 37·2	21·5, 21·6
Furfural content of derived hemicellulose	17·4	8·43

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* 'Monatshefte f. Chemie,' vol. 46, p. 31 (1925); and Fuchs, *loc. cit.*, p. 32

Summary.

The substances which accompany cellulose in the cell-walls of plants may be divided into three classes: (i) lignins, (ii) hemicelluloses, (iii) pectins. The products belonging to the two latter classes are formed by conjugation of the sugar acids (glycuronic and galacturonic acids) with sugars. These acids are, using Ling's suggestion, designated "uronic acids," and the hemicelluloses and pectins appear therefore to belong to a distinct chemical group, for which the name "polyuronides" is suggested. The pectins contain a much larger uronic acid content than do the hemicelluloses, while the uronic acids are absent from the lignins.

Lignified tissues contain lignins and hemicelluloses in relatively large amounts, with only traces (if any) of pectins. Non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin.

It is found that pectins undergo decarboxylation on treatment with weak alkaline solutions, even at room temperature. On this treatment they yield, amongst other products, hemicelluloses, which still contain uronic groups, but which resist decarboxylation on treatment with alkalis, and which resemble in all respects the hemicelluloses isolated directly from timbers.

These results indicate that decarboxylation takes place when plant tissues lignify. No direct connection has yet been traced between the pectins and lignins.

The Plasticity of Wool.

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Introduction.

1. Shorter (1) has shown that the behaviour of wool under tension can be simulated by the Poynting and Thomson (2) model, consisting of two springs connected in series, one spring being free and the other damped by immersion in a viscous medium. The same model has been used more recently by Poole (3) to illustrate the behaviour of gelatin gels. Thus a wool fibre, when subjected to tension, shows an immediate extension, followed by slow creep, and Shorter considers that "the explanation of this is not, as might be supposed, that the elastic elements are showing plastic yield, but that the fibre contains elastic elements with very different degrees of damping." Similarly, the elastic relaxation which occurs when a fibre is held stretched to a definite length is regarded, not as the disappearance of strain owing to molecular re-adjustment, but as "the transference of a state of strain from lightly damped to highly damped elements." (1)

When dry, extended wool fibres tend to return only very slowly to their original length, but immersion in water produces rapid and complete recovery. In terms of the model, this observation is explained by assuming the viscosity of the colloid medium to be reduced by water absorption. Its viscosity is also reduced by the mechanical disturbance associated with extension and contraction, but if a period of rest be allowed between two successive extensions, the fibre is found to be completely unaltered by extension. Shorter's observations were carried out at 68 per cent. relative humidity, and it is clear that under these conditions wool is perfectly elastic.

In contradistinction to the behaviour of relatively dry wool, the author has been able to show that wool fibres in water are imperfectly elastic. The imperfection is of two kinds, attributable to the plasticity and rupture of elements which are elastic when dry.

Experimental.

2. The plasticity of wool in water can be illustrated in a number of ways, but the most striking proof is obtained by a study of the stress-strain relationships of fibres before and after being held stretched to a definite length in water.

The general method of experiment was as follows: a 5-cm. length of fibre, attached by means of sealing wax to two glass hooks, was stretched between a fixed base and one arm of a balance. Load was applied at a constant rate by running water through a calibrated jet into a counterpoised beaker carried by the right-hand arm. In view of the high extensibility of wool when wet, and the weakness of some of the finer fibres, the left and right-hand arms of the balance had effective lengths in the ratio 3 : 1. The fibre was immersed in a vessel of water surrounded by a thermostat at 25° C., and extension followed by observing the upper end of the fibre through a travelling microscope sensitive to 0·05 mm. (0·1 per cent. of the original length). The load-extension curve was determined up to 30 per cent. extension, and the fibre held stretched at this point for a definite time. On being released, it returned exactly to the original length, and was allowed to rest unstretched in water for 24 hours. The load-extension curve was then re-determined.

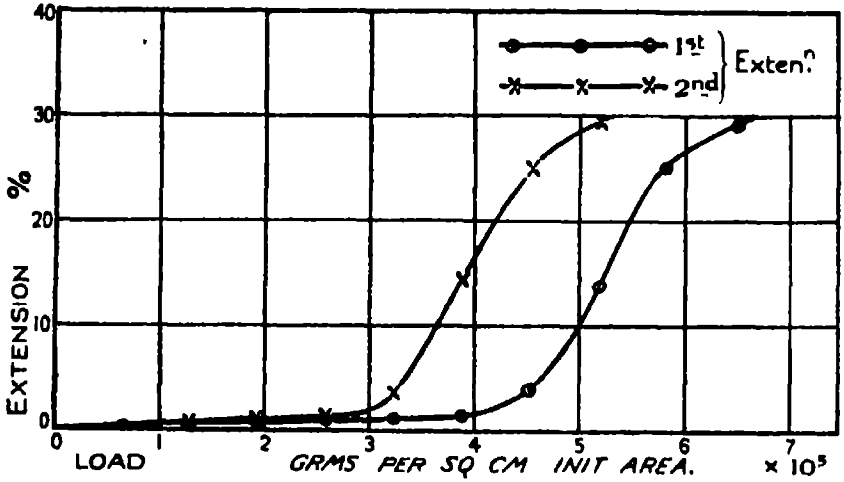


Fig. 1.—Typical Load-extension Curves.

Two typical curves for a fibre held stretched four hours are shown in fig. 1, and indicate a marked change of elastic properties. Furthermore, the change is permanent, for an interval of 18 days between successive extensions failed to induce recovery. If plastic flow is responsible for the greater ease of extension, the separation of the two load-extension curves should increase with the time for which fibres are held stretched. Accordingly, a number of experiments were made with fibres held stretched for times varying between 30 and 1143 minutes. From the curves, the amounts of energy necessary to extend each fibre 30 per cent. of its length before and after stretching were evaluated.

The data given in Table I have been calculated for a unit cube of wool by correcting for the measured cross-sectional area of each fibre. They show a continuous reduction in the energy necessary to extend fibres with increasing time of stretching, and afford a convincing proof that part, at least, of the wool fibre is truly plastic in water.

Table I.

E_1 = Initial energy necessary to extend fibres 30 per cent.

E_2 = Energy necessary to extend fibres 30 per cent. after stretching.

Time held stretched.	E_1.	E_2.	$E_1 - E_2$.	$\frac{(E_1 - E_2)}{E_1} \times 100$.
Mins.	gramme cms. per c.c. × 10^6.			
30	1·529	1·429	0·100	6·5
62	1·478	1·353	0·125	8·5
92	1·555	1·376	0·179	11·5
161	1·539	1·244	0·295	19·2
210	1·627	1·277	0·350	21·5
240	1·574	1·189	0·385	24·4
240	1·536	1·158	0·378	24·6
300	1·479	1·084	0·395	26·7
1143	1·494	0·891	0·603	40·3

Corresponding experiments were made at lower humidities. As before, the load-extension curves were determined in water, but the fibres were held stretched in atmospheres at 90 per cent. and 75 per cent. relative humidity. The results given in Table II include those for 100 per cent. humidity for purposes of comparison, and indicate that wool is not markedly plastic except at humidities approaching saturation.

Table II.

Relative Humidity.	Time held stretched.	E_1.	E_2.	$E_1 - E_2$.	$\frac{E_1 - E_2}{E_1} \times 100$.
Per cent.	Mins.	gramme cms. per c.c. × 10^6.			
100	300	1·479	1·084	0·395	26·7
90	303	1·389	1·270	0·119	8·6
75	323	1·620	1·532	0·088	5·4

3. The second type of imperfection shown by wool in water is due to the actual rupture of elastic elements by extension, but even when such breakage occurs the fibre retains its ability to return exactly to the original length. A measure of the degree of breakdown at each extension can therefore be obtained

by determining the reduction in the energy necessary to extend fibres a given amount after an equal previous extension under the same conditions. The determinations must be made under such conditions that plastic flow is absent, and to conform with this requirement fibres were stretched *rapidly* in water at a lower temperature than before, 18° C. The actual rate of loading was $1 \cdot 4 \times 10^5$ gms./sq. cm./minute, and is sufficient to break fibres at about 50 per cent. extension in about 12 minutes. The data of Table I show that the amount of plastic flow which can occur in this maximum time is negligibly small.

The method of experiment was similar to that already described, with the one exception that the fibres were not held stretched to a definite length, but were allowed to contract immediately the required extension had been attained. Once-extended fibres showed a slight partial recovery of elastic properties on standing, but all possible recovery was complete within 24 hours. The amounts of energy necessary to extend fibres a certain degree initially, and after an equal previous extension, were again graphically evaluated. The complete results for a number of different extensions appear in Table III.

Table III.

E_1 = Initial energy necessary to extend fibres.

E_2 = Energy necessary to extend fibres after an equal previous extension.

Percentage Extension.	E_1.	E_2.	$E_1 - E_2$.	$\frac{E_1 - E_2}{E_1} \times 100$.
	gramme cms. per c.c. $\times 10^8$.			
35 4	1·927	1·894	0·033	1·70
38 4	2 389	2 326	0·063	2·64
40 4	2·797	2·610	0·187	6·69
40·5	2·596	2·422	0·174	6·71
45·7	2·919	2·566	0 353	12·1
46 3	3·494	3·082	0·412	11·8
49 7	3 494	2·997	0·497	14·2
50 3	3·668	3·065	0·603	16 4
51·6	4 407	3·474	0·933	21·1
54·6	4 473	3 416	1·057	23·6

The most important deduction to be made from these observations is that Cotswold wool fibres, when extended rapidly in water, experience no permanent alteration below 34 per cent. extension. Beyond this point, the degree of elastic imperfection produced by actual rupture of elements within the fibre increases continuously with the extension attained. This form of imperfection is present at lower humidities than saturation, but to a smaller degree. For example, at 75 per cent. relative humidity, the amounts of energy necessary to extend fibres 40 per cent. of their length initially, and after an equal previous extension, were 4·206 and 4·082 gm. cms. per c.c. The reduction in energy is only 2·94 per cent. of the original energy necessary, and in view of the fact that it is impossible at this humidity to extend fibres far beyond 40 per cent. without actual rupture occurring, the degree of imperfection in " dry " wool fibres must be regarded as relatively small.

4. The two forms of elastic imperfection in wet wool necessitate some modification of Shorter's theory. This was developed for wool in the semi-dry and perfectly elastic condition, and it is clear that no arrangement of springs in series can explain the newly-discovered ability of wool fibres always to return to their original length in spite of the plasticity and rupture of internal elements. The most satisfactory explanation is that the fibre consists of a number of elements arranged in parallel, all of which are elastic at low humidities, but some of which become plastic at high humidities. So long as any of the elements remain perfectly elastic, the fibre as a whole will possess perfect elasticity of form. By introducing the microscopic observations of Nathusius (4) and Mark (5), it is possible to give this modified theory increased precision. Nathusius found that the constituent cells of wool and hair consist of a cell wall enclosing a fibrillar structure, which is not arranged haphazardly, but has a tendency to lie preferentially along the axis of the fibre. More recently, Mark has been able to show that the extension of wool fibres takes place by direct stretching, without slip, of the constituent cells. Evidently then, the elastic properties of the fibre as a whole are those of the single cell.

The preferential arrangement of fibrillæ along the axis of the fibre is confirmed by the form and interpretation of the stress-strain diagram for wool (fig. 1). This falls naturally into three sections : a period of slow extension, over which Hooke's Law is approximately obeyed, followed by a period of rapid extension from 2 per cent. to 30 per cent., which is again succeeded by a period of slow extension. From the present point of view, the most important section of the curve is that of rapid extension. The fact that extension is most rapid over the range where elastic imperfection is entirely absent, makes it probable that such extension occurs by rotation of fibrillæ. When all the fibrillæ have been drawn into the line of application of stress, extension is again retarded.

But Poole (3) has shown that the extension realised by rotation in the case of a haphazardly arranged fibrillar structure is of the order of 60 per cent. The fibrillar structure of wool must therefore be preferentially arranged along the axis of the fibre. This arrangement is what would be expected from the mode of formation and growth of the fibre. It is known that the cells which go to form wool and hair are originally spherical in form, but as they are forced up the shaft of the fibre deformation to either scale or spindle-shape takes place. The contents of the origoial cell are probably gelatinous in character, but during deformation there will be a tendency to develop fibrillæ parallel to the length, rather than at an angle to the length. Keratinisation serves to conserve the arrangement.

In order to explain the elastic properties of wool it is necessary to assume only that the cell wall is perfectly elastic in water. This assumption is not entirely unsupported by experimental evidence, and there is reason, on biological grounds, to expect the cell wall to differ in constitution from the enclosed fibrillæ, which are to be regarded as elastic when dry, but plastic when wet. The wool fibre as a whole, therefore, consists of an elastic continuous cellular framework, enclosing a fibrillar structure, which is arranged preferentially along the axis of the fibre.

5. On the above theory, two forms of elastic relaxation are to be expected when a wool fibre is held stretched to a definite length. The first is of the kind illustrated by the Poynting and Thomson model, and is due to the impedence of fibrillar rotation by frictional forces within the cell, while the second is attributable to molecular re-arrangement within the plastic fibrillæ. The quantitative study of the plasticity of different wools and hairs, and its dependence on humidity and chemical treatment, was attempted by determining the rate of decay of tension in fibres held stretched to a fixed length.

The apparatus used in this section of the investigation requires more detailed description, and is shown in front and end elevation in fig. 2. It consists essentially of a heavy brass base carrying two vertical pillars, midway between which turns a calibrated brass screw of 1 mm. pitch. A traveller, T, carrying a calibrated steel spring, S, slides along the pillars, and is actuated by turning the screw. The number of turns made by the latter is indicated by the pointer, P, which moves over a dial graduated in quarter turns. Below the spring, S, is a rectangular glass tank cemented to the metal base, through which the plunger, L, carrying a hook at its upper end, is operated. A glass lid, cut in half, closes the tank, with the exception of a pinhole in the centre immediately above the hook of the plunger.

Humidity control within the tank was effected by sulphuric acid solutions contained in 3-tier paraffin-wax troughs, arranged on either side of the plunger. For observations at 100 per cent. relative humidity, the tank was filled with distilled water. The whole apparatus was built into a thermostat at 25° C., the water of which came almost to the level of the top of the glass tank.

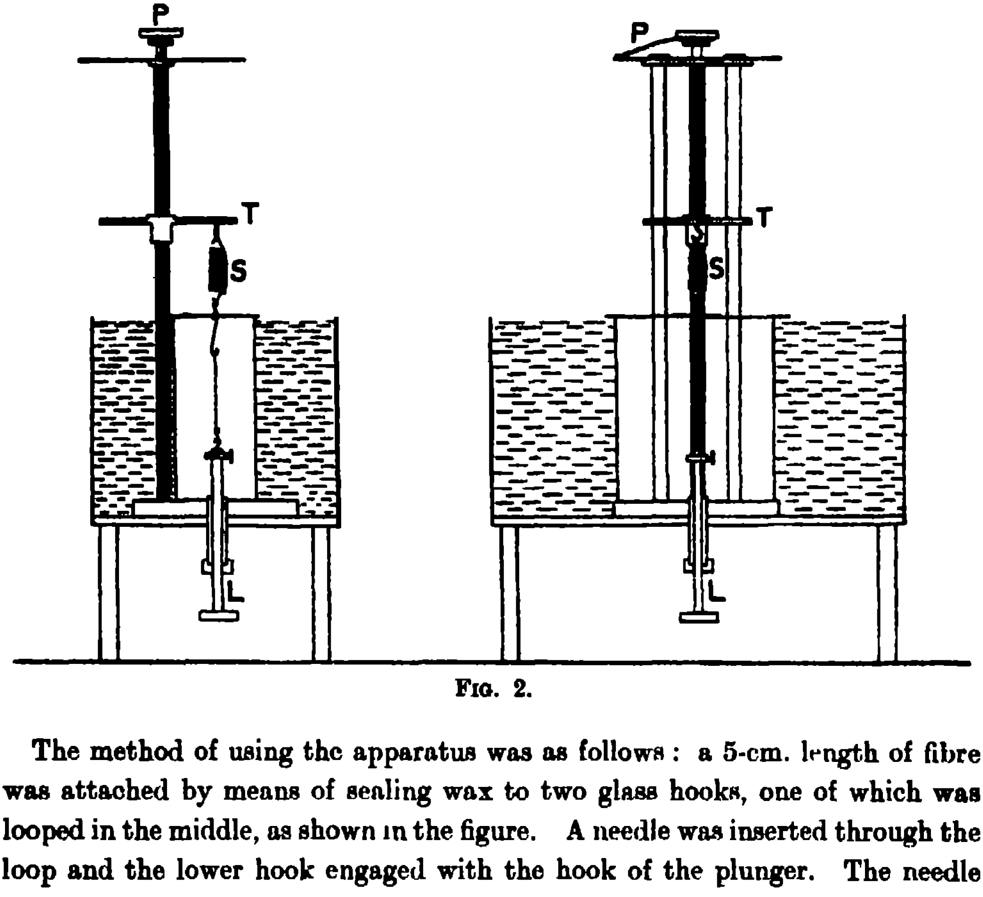


Fig. 2.

The method of using the apparatus was as follows: a 5-cm. length of fibre was attached by means of sealing wax to two glass hooks, one of which was looped in the middle, as shown in the figure. A needle was inserted through the loop and the lower hook engaged with the hook of the plunger. The needle was then rested on the lid of the tank and the fibre just drawn taut by gradually withdrawing the plunger. The spring, S, was then lowered and engaged with the upper hook, which moved freely upwards within the pinhole in the lid. After measuring its unstretched length by means of a reading microscope, the fibre was extended rapidly by withdrawing the plunger to its extremity. The spring was then extended by raising the traveller until the upper hook lifted slightly from the needle. If the system were then left untouched, the fibre would extend and the spring contract.

To obviate extension of the fibre, the spring was caused to contract by turning the pointer, P, in such a way that the fibre, as observed through a microscope, was held stretched at constant length. Originally, the pointer was operated continuously, and the rate of decay of tension determined by measuring the length of the spring at intervals. Later, it was found more convenient to make half turns of the pointer, and observe the times at which the extremity of the upper glass hook attached to the fibre made apparent contact with a certain graduation of the eyepiece micrometer. The contraction caused by the half turn of the screw was absorbed mainly by the spring, the length of the fibre being altered by only one part in 500. At the end of the experiment, the length of the spring was measured by the aid of a travelling microscope, and its length at any previous time calculated from the known number of half-turns which had been made. The stretched length of the fibre was also measured.

The Influence of Relative Humidity on the Elastic Relaxation of Extended Wool Fibres.

6. Observations were made at 25, 50, 75 and 100 per cent. relative humidity. At the lowest humidity it is not possible to extend fibres far beyond 30 per cent. extension without rupture occurring, and in consequence extension was limited to this amount (approximately) in all cases. The general trend of elastic relaxation at each humidity is shown by the following typical figures for Cotswold fibres at 25° C. :—

Table IV.

25 per cent. Relative Humidity.
Fibre Diameter : 45·1 μ.
Extension : 30 per cent.

Time.	Tension.
Mins.	Grammes per cm.^{2}
1·75	$16{\cdot}07 \times 10^5$
2·70	$15{\cdot}72 \times 10^5$
4·45	$15{\cdot}39 \times 10^5$
8 35	$15{\cdot}06 \times 10^5$
16·10	$14{\cdot}72 \times 10^5$
32 05	$14\ 39 \times 10^5$
59 75	$14{\cdot}06 \times 10^5$
170	$13\ 55 \times 10^5$
1306	$12\ 57 \times 10^5$

50 per cent. Relative Humidity.
Fibre Diameter : 43·3 μ.
Extension : 28·9 per cent.

Time.	Tension.
Mins.	Grammes per cm.^{2}
1·50	$13{\cdot}63 \times 10^5$
2·73	$13{\cdot}28 \times 10^5$
3·75	$13{\cdot}10 \times 10^5$
5 57	$12{\cdot}91 \times 10^5$
7·95	$12{\cdot}73 \times 10^5$
12 15	$12{\cdot}55 \times 10^5$
17·60	$12{\cdot}37 \times 10^5$
27·47	$12{\cdot}19 \times 10^5$
42·80	$12{\cdot}01 \times 10^5$
65·20	$11{\cdot}82 \times 10^5$
95·90	$11{\cdot}64 \times 10^5$
210	$11{\cdot}28 \times 10^5$
280	$11{\cdot}09 \times 10^5$

Table IV—*continued*.

75 per cent. Relative Humidity.
Fibre Diameter: 45·8 μ.
Extension: 27·2 per cent.

Time.	Tension.
Mins.	Grammes per cm.²
1·20	10·39 × 10^5
2·00	10·07 × 10^5
4·35	9·74 × 10^5
9·65	9·41 × 10^5
21·50	9·09 × 10^5
46·55	8·76 × 10^5
99·65	8·44 × 10^5
200	8·11 × 10^5
1339	6·95 × 10^5

100 per cent. Relative Humidity.
Fibre Diameter: 46·6 μ.
Extension: 29 per cent.

Time.	Tension.
Mins.	Grammes per cm.²
1·05	6·05 × 10^5
1·75	5·89 × 10^5
2·90	5·74 × 10^5
4·95	5·58 × 10^5
7·80	5·42 × 10^5
11·50	5·26 × 10^5
16·60	5·11 × 10^5
22·55	4·95 × 10^5
30·25	4·80 × 10^5
39·20	4·64 × 10^5
50·35	4·49 × 10^5
62·15	4·33 × 10^5
76·60	4·17 × 10^5
1143	1·97 × 10^5

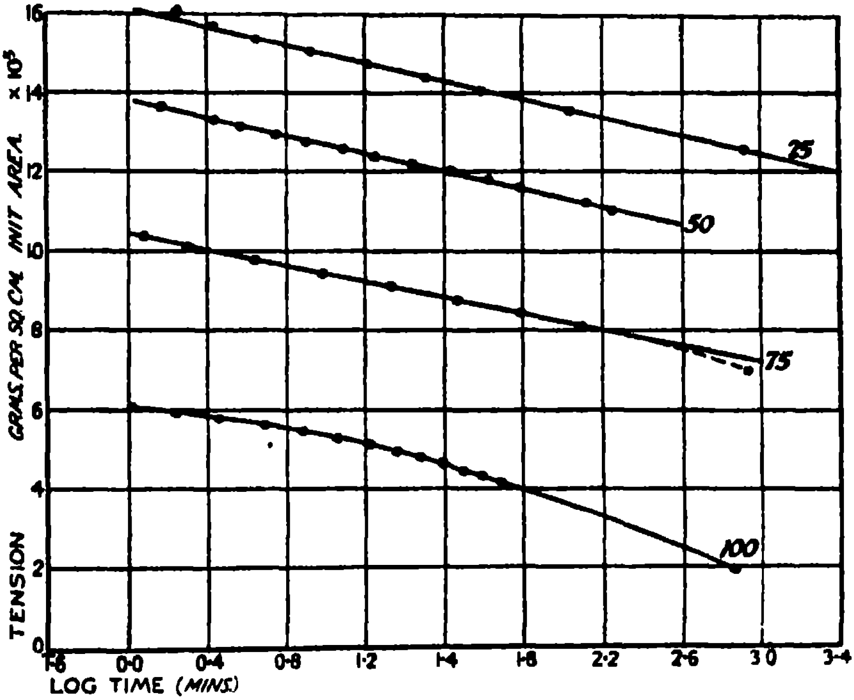


Fig. 3.—Decay of tension at humidities 25, 50, 75 and 100 per cent.

In fig. 3 tension is graphed against logarithm of time, illustrating the existence of a linear relationship between the two quantities, except at

100 per cent. humidity, where the deviation is well marked. The same expression,

$$T = a - k \log (t + c),$$

where T = stress at any time t, and a, k and c are constants, has been used by Trouton and Rankine (6) for the rate of decrease of stress in lead wires held stretched at constant length. The complete failure of the equation in the case of wool stretched at high humidities must be due to the incidence of fibrillar plasticity.

It is possible to fit all the results of Table IV to an equation of the type

$$T = c + ae^{-kt^n},$$

where T = tension at time t, and c, a, k and n are constants. This expression, due originally to Kohlrausch, (7) has been employed by Peirce (8) with considerable success in the case of the decay of torsional resistance in cotton fibres at constant twist. Unfortunately, the value of the exponent "n" is different for wool at high and low humidities, and for wool treated chemically in a variety of ways. The equation cannot, therefore, be conveniently used for the comparison of the fibrillar plasticity of different wools under different conditions. In consequence, the degree of departure from the Trouton and Rankine equation has been adopted here as a measure of fibrillar plasticity.

The Plasticity of Different Wools and Animal Hairs.

7. Different materials were compared in water where plasticity is most marked. After immersion in water at 25° C. for 30 minutes, the fibres were rapidly extended 40 per cent. of their length, and the rate of decay of tension at constant length determined. The plasticity of different fibres, even from the same sample of wool, is by no means uniform, and comparison of different materials can only be made by averaging a number of determinations. The only possible method of averaging and comparing results seems to be to measure the time taken for the initial tension to decay to half value. The set of ten observations made with Cotswold wool are recorded in Table V to indicate the degree of variation encountered.

Table V.—Cotswold Wool.

Percentage Extension.	Half-tension Time.
	Mins.
42·4	98
41·1	95
38·6	82
40·0	120
39·1	108
38·3	110
42·1	170
39·1	122
39·3	118
40·4	120

The mean values of the half-tension times for four kinds of fibre are given in Table VI. It should be mentioned that comparison has been limited to these fibres because they were extremely uniform in diameter. Other more irregular wools have been studied but the results have no real significance, in the sense that they are true only for wool as a fibre and not as a substance. The values given in the table express real differences in the composition of the fibres, and are independent of geometrical properties.

Table VI.

Kind of Fibre.	Extension.	Half-tension Time.
	Per cent.	Mins.
Human Hair	39·5	233
Mohair	39·6	156
Cotswold Wool	40·0	114
Australian Leicester Wool	39·8	88

The Influence of Chemical Reagents on the Fibrillar Plasticity of Wool.

8. Although wool is treated with a variety of chemical reagents during its passage from the raw material to the finished fabric, their effect on its elastic properties has never been studied. From the present point of view, however, the most important aspect of the action of chemical reagents is the information derived regarding the development of plasticity in water. The nature of each reaction is not discussed separately because the theory to be formulated is based on the cumulative evidence of all the reactions.

(*a*) *Inorganic Acid.*—Single wool fibres were immersed in large volumes of 1·200 N/100 and 1·015 N/10 hydrochloric acid solutions at 25° C. for 24 hours and then stretched about 40 per cent. of their length in the solution with which they had come to equilibrium. The rates of decay of tension were determined, giving results of the type shown below (Table VII).

Table VII.—Cotswold Wool.

Fibre Diameter : 35·9 μ.
Extension : 39·6 per cent.
1·200 N/100 HCl.

Time.	Tension.
Mins.	Grammes per sq. cm.
0·68	$9\cdot06 \times 10^5$
1·15	$8\cdot80 \times 10^5$
2·05	$8\cdot53 \times 10^5$
3·33	$8\cdot26 \times 10^5$
5·67	$8\cdot00 \times 10^5$
8·25	$7\cdot74 \times 10^5$
12·95	$7\cdot47 \times 10^5$
18·35	$7\cdot21 \times 10^5$
27·30	$6\cdot95 \times 10^5$
37·95	$6\cdot69 \times 10^5$
55·25	$6\cdot41 \times 10^5$
124·7	$5\cdot62 \times 10^5$

Fibre diameter : 35·6 μ.
Extension : 38 per cent.
1·015 N/10 HCl.

Time.	Tension.
Mins.	Grammes per sq. cm.
0 85	$10\cdot03 \times 10^5$
1·27	$9\cdot77 \times 10^5$
1·93	$9\cdot50 \times 10^5$
3·00	$9\cdot24 \times 10^5$
4·65	$8\cdot96 \times 10^5$
7·55	$8\cdot70 \times 10^5$
11·50	$8\cdot43 \times 10^5$
19 15	$8\cdot16 \times 10^5$
28·40	$7\cdot89 \times 10^5$
44·95	$7\cdot62 \times 10^5$
65·75	$7\cdot35 \times 10^5$
152·6	$6\cdot71 \times 10^5$

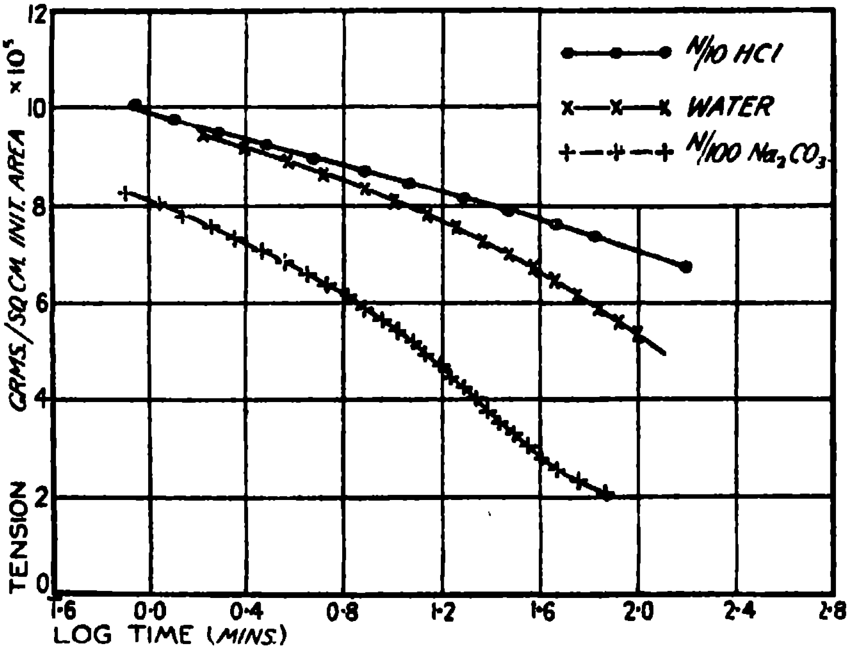


Fig. 4.—Decay of tension in various media.

In fig. 4 tension is graphed against logarithm of time for the fibre in equilibrium with 1·015 N/10 hydrochloric acid, and for an untreated fibre extended

38·6 per cent. of its length in water at 25° C. It is evident that the plasticity of wool, as evidenced by the rate of decay of tension, is considerably less in acid solution than in water.

(*b*) *Alkali.*—The effect of alkali on the plasticity of wool was determined by studying the rate of decay of tension in 0·99 N/100 sodium carbonate solution at 25° C. Single fibres were immersed in large volumes of the solution for 24 hours, and elastic relaxation at about 40 per cent. extension studied *in situ.* Typical results are given below (Table VIII), and tension is plotted against the logarithm of time in fig. 4. From the latter it is clear that the plasticity of wool is greatly increased, even in very dilute alkali.

Table VIII.

Fibre Diameter: 37·9 μ. Extension: 39·8 per cent. 0·99 N/100 Sodium Carbonate.

Time.	Tension.	Time.	Tension.
Mins.	Grammes per cm.^{2}	Mins.	Grammes per cm.^{2}
0·80	$8·26 \times 10^5$	13·45	$4·94 \times 10^5$
1·10	$8·02 \times 10^5$	15·32	$4·70 \times 10^5$
1·38	$7·79 \times 10^5$	17·22	$4·46 \times 10^5$
1·80	$7·55 \times 10^5$	19·50	$4·22 \times 10^5$
2·25	$7·31 \times 10^5$	21·65	$3·98 \times 10^5$
2·93	$7·07 \times 10^5$	24·40	$3·75 \times 10^5$
3·55	$6·84 \times 10^5$	27·00	$3·51 \times 10^5$
4·45	$6·60 \times 10^5$	30·62	$3·28 \times 10^5$
5·28	$6·36 \times 10^5$	34·30	$3·04 \times 10^5$
6·43	$6·12 \times 10^5$	39·80	$2·80 \times 10^5$
7·50	$5·89 \times 10^5$	46·20	$2·56 \times 10^5$
8·93	$5·65 \times 10^5$	56·30	$2·32 \times 10^5$
10·25	$5·41 \times 10^5$	71·25	$2·09 \times 10^5$
11·95	$5·17 \times 10^5$		

The half-tension time for the preceding results is 11·7 minutes. It is well known that wool is disintegrated in dilute caustic soda solution, but its increased plasticity in N/100 sodium carbonate is not due to any permanent chemical decomposition, for a similar fibre to the preceding, left 24 hours in the solution and then washed in running water for 1 hour, gave a half-tension time of 176 minutes in water.

(*c*) *Neutral Salts.*—By analogy with the properties of proteins in general, it is to be expected that the swelling of wool in water will be increased by the presence of neutral salts in solution. The plasticity of wool should be greater in a solution of common salt than in water, and still greater in a solution of potassium sulphocyanide. Elastic relaxation was studied in N/10 NaCl solution and in a saturated solution of KCNS at 25° C., but no significant alteration of plasticity could be detected. Typical data are given in Table IX, and the half-tension times were 104 minutes (NaCl) and 131 minutes (KCNS).

Table IX.

Fibre Diameter : 39·3 μ. Extension : 37·5 per cent. N/10 NaCl solution.		Fibre Diameter : 42·5 μ. Extension : 42·1 per cent. Saturated KCNS solution.	
Time.	Tension.	Time.	Tension.
Mins.	Grammes per cm.^{2}	Mins.	Grammes per cm.^{2}
0·88	$11\cdot00 \times 10^5$	1·12	$8\cdot74 \times 10^5$
1·15	$10\cdot80 \times 10^5$	1·35	$8\cdot56 \times 10^5$
1·50	$10\cdot57 \times 10^5$	1·68	$8\cdot37 \times 10^5$
2·07	$10\cdot36 \times 10^5$	2·15	$8\cdot18 \times 10^5$
2·80	$10\cdot12 \times 10^5$	2·85	$7\cdot99 \times 10^5$
3·70	$9\cdot91 \times 10^5$	3·65	$7\cdot80 \times 10^5$
4·80	$9\cdot68 \times 10^5$	4·85	$7\cdot61 \times 10^5$
6·38	$9\cdot47 \times 10^5$	6·48	$7\cdot42 \times 10^5$
8·17	$9\cdot24 \times 10^5$	8·53	$7\cdot24 \times 10^5$
10·40	$9\cdot03 \times 10^5$	11·15	$7\cdot05 \times 10^5$
13·15	$8\cdot80 \times 10^5$	14·85	$6\cdot86 \times 10^5$
16·35	$8\cdot59 \times 10^5$	19·20	$6\cdot67 \times 10^5$
20·40	$8\cdot36 \times 10^5$	43·40	$6\cdot10 \times 10^5$
30 00	$7\cdot92 \times 10^5$	55 30	$5\cdot91 \times 10^5$
43·45	$7\cdot48 \times 10^5$	72 20	$5\cdot73 \times 10^5$
50 65	$7\cdot26 \times 10^5$	1093	$3\cdot18 \times 10^5$
60	$7\cdot04 \times 10^5$		
1136	$1\ 46 \times 10^5$		

(*d*) *Colour Acids.*—A sample of Cotswold wool was dyed with 2 per cent. of its weight of the acid dye tartrazine, in presence of sulphuric acid and sodium sulphate. The sulphuric acid absorbed by the wool was removed by washing in running water and elastic relaxation of extended fibres then studied in water at 25° C. The half-tension times of a number of fibres varied from 360 to 1750 minutes, and, although in every case the time was greater than for untreated wool, the irregularity of results is surprising. It must be referred to the irregular absorption of dye by different fibres. Typical results for a fibre having a half-tension time of 496 minutes are given in Table X, together with data for an untreated fibre in water at 25° C. for comparison.

Table X.

Fibre Diameter: 37 μ.
Extension: 39 per cent.
Dyed with Tartrazine.

Time.	Tension.
Mins.	Grammes per cm.²
0·75	10·08 × 10^5
1·15	9·82 × 10^5
2·05	9·57 × 10^5
3·25	9·33 × 10^5
5·50	9·08 × 10^5
8·55	8·83 × 10^5
13·20	8·58 × 10^5
18·95	8·33 × 10^5
26·95	8·09 × 10^5
36·85	7·83 × 10^5
49·80	7 59 × 10^5
66·35	7·34 × 10^5
115·95	6·84 × 10^5
187·00	6 34 × 10^5

Fibre Diameter: 35·2 μ.
Extension: 38·6 per cent.
Untreated.

Time.	Tension.
Mins.	Grammes per cm.²
1·65	9·46 × 10^5
2 45	9·18 × 10^5
3·73	8·90 × 10^5
5·15	8·63 × 10^5
7·55	8·35 × 10^5
10·00	8·08 × 10^5
13·70	7·80 × 10^5
17·60	7·53 × 10^5
23·00	7·25 × 10^5
29·05	6·98 × 10^5
36·65	6·70 × 10^5
44·40	6·43 × 10^5
55 20	6·16 × 10^5
66 60	5·88 × 10^5
81 35	5 60 × 10^5
97 00	5·33 × 10^5

It is obvious that the plasticity of wool in water is reduced by combination with an acid dye. Similar experiments were carried out with Cotswold wool dyed with picric acid, their object being to dispense with the use of sulphuric acid and to discover whether combination of wool with large quantities of colour acid was capable of eliminating plasticity. One gramme of wool was boiled for 40 minutes with 0·5 gramme of picric acid dissolved in 500 c.c. of water. The elastic relaxation of the heavily dyed fibres was studied in water at 25° C. Far more uniform results were obtained than before, and in every case plasticity was almost entirely eliminated. During the time the fibre was held stretched in water, however, picric acid was gradually washed out of the wool and, when sufficient had been removed, plasticity again developed. This phenomenon is well shown by the data of Table XI.

Table XI.—Fibre Diameter: 46·6 μ. Extension: 38·3 per cent. Dyed with Picric Acid.

Time.	Tension.	Time.	Tension.
Mins.	Grammes per cm.^{2}	Mins.	Grammes per cm.^{2}
1·00	$9·57 \times 10^5$	26·55	$7·86 \times 10^5$
1·35	$9·43 \times 10^5$	35·35	$7·69 \times 10^5$
1·95	$9·26 \times 10^5$	46·40	$7·54 \times 10^5$
2·62	$9·11 \times 10^5$	59·90	$7·38 \times 10^5$
3·55	$8·95 \times 10^5$	76·55	$7·23 \times 10^5$
4·63	$8·80 \times 10^5$	93·70	$7·06 \times 10^5$
6·25	$8·63 \times 10^5$	114·85	$6·92 \times 10^5$
8·00	$8·49 \times 10^5$	135·40	$6·75 \times 10^5$
10·70	$8·31 \times 10^5$	333·00	$5·81 \times 10^5$
14·47	$8·17 \times 10^5$	1587	$2·05 \times 10^5$
20·05	$8·01 \times 10^5$	5748	$1·49 \times 10^5$

(*e*) *Formaldehyde and Quinone.*—By the action of formaldehyde, proteins are converted into compounds which do not swell or dissolve to any marked degree in water. The reduction in swelling power is equivalent to a reduction in plasticity, and it is to be expected that formaldehyde will reduce the plasticity of wool in water. The action of formaldehyde on wool was studied by immersing fibres in 40 per cent. formaldehyde solution at 25° C., and determining the rate of decay of tension in the same solution. It was not found possible to extend fibres beyond 36 per cent. without rupture, and the data given in Table XII are not therefore comparable with those given previously.

Table XII.—Fibre Diameter: 37·7 μ. Extension: 35·4 per cent. 40 per cent. Formaldehyde Solution.

Time.	Tension.
Mins.	Grammes per cm.^{2}
0·97	$10·58 \times 10^5$
1·55	$10·32 \times 10^5$
2·60	$10·10 \times 10^5$
4·60	$9·83 \times 10^5$
8·15	$9·61 \times 10^5$
15·45	$9·36 \times 10^5$
26·55	$9·13 \times 10^5$
45·25	$8·89 \times 10^5$
75·65	$8·64 \times 10^5$
190 55	$8·16 \times 10^5$

A linear relationship holds between tension and the logarithm of time, indicating the disappearance of fibrillar plasticity. A similar reduction of plasticity was obtained when wool was treated with quinone in aqueous solution.

(*f*) *Nitrous Acid.*—When wool is treated with nitrous acid, nitrogen is evolved by the replacement of amino by hydroxyl groups, and at the same time nitroso compounds are formed (9) by reaction with imido groups. The product of the reaction is commonly known as "diazotised wool." A sample of "diazotised wool" was prepared by immersing Cotswold wool in a solution of sodium nitrite acidified with acetic acid. After 24 hours the wool was removed and washed free from acetic acid in running water. The rate of decay of tension in extended fibres was determined in water at 25° C., typical data being given in Table XIII.

Table XIII.—Fibre Diameter: 37·7 μ. Extension: 40·7 per cent. Nitrous Acid.

Time.	Tension.
Mins.	Grammes per cm.^{2}
1 15	$10{\cdot}78 \times 10^5$
1·90	$10{\cdot}51 \times 10^5$
3·25	$10{\cdot}28 \times 10^5$
5·35	$10\ 02 \times 10^5$
9·05	$9{\cdot}79 \times 10^5$
13·90	$9{\cdot}55 \times 10^5$
22·10	$9{\cdot}29 \times 10^5$
32 10	$9\ 05 \times 10^5$
48·85	$8{\cdot}81 \times 10^5$
66 00	$8{\cdot}56 \times 10^5$
98·65	$8{\cdot}31 \times 10^5$
1476	$6{\cdot}24 \times 10^5$
2850	$5{\cdot}11 \times 10^5$

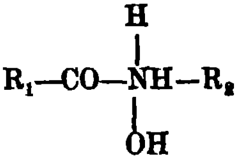
The results obtained with different fibres were remarkably uniform, the half-tension time being more than 24 hours, and the reduction in plasticity extremely well marked.

Theoretical.

9. The most striking feature of the preceding study of the action of chemical reagents on wool is the generalisation to which it gives rise: that those reagents which react or combine with amino or imido groups without causing hydrolysis reduce the plasticity of wool in water. It is well known that wool treated with formaldehyde, acid dyes or nitrous acid has a reduced adsorptive capacity for water. Trotman (10), for example, found that at about 65 per cent. relative humidity the adsorptive capacities of wool combined with paraformaldehyde and formaldehyde were only 59 per cent. and 77 per cent. respectively of that of untreated wool. There is therefore every indication that plasticity

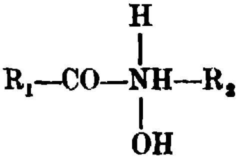
is induced by the affinity of amino and imido groups for water, and is prevented by converting these groups into forms incapable of water adsorption.

The characteristic linkages between the constituent amino acids and molecules of wool are the — CO — NH — or peptide groups, which are not all equally stable in water. Those produced by the polymerisation of wool during drying will be readily hydrolysed by water, while those which form an integral part of the structure of the wool " molecule " can only be hydrolysed by the action of more powerful reagents, such as caustic soda. When water is adsorbed by dry wool, unstable compounds of the type



$$\mathrm{R_1{-}CO{-}\underset{\underset{\textstyle OH}{|}}{\overset{\overset{\textstyle H}{|}}{N}}H{-}R_2}$$

are probably formed, and the field of force surrounding each group is reduced. Free amino groups must form similar compounds, and for these two reasons *at least* the resistance of wool fibres to extension will decrease with increasing water adsorption, as is found by experiment (Table IV). At high humidities, and especially in presence of liquid water, there will be an increasing tendency for adsorption compounds of the type



$$\mathrm{R_1{-}CO{-}\underset{\underset{\textstyle OH}{|}}{\overset{\overset{\textstyle H}{|}}{N}}H{-}R_2}\quad \text{to transform into}\quad \mathrm{R_1{-}COO{-}NH_3{-}R_2},$$

followed by actual division of the molecule into R_1COOH and R_2NH_2 in the case of the less stable linkages. Each of these transformations will produce a corresponding reduction in the affinity between molecules and a greater tendency to plasticity.

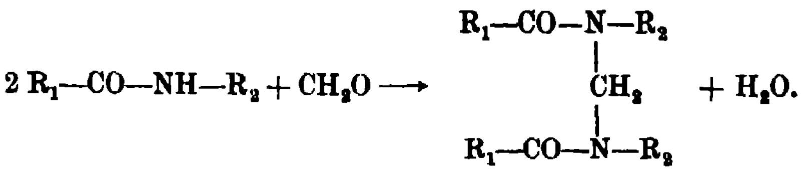
The succession of changes thus postulated is initiated by adsorption of water by trivalent nitrogen, and it is evident that any reagent which prevents water adsorption will inhibit further degradation and plasticity. Thus in solutions of inorganic acid, trivalent nitrogen combines with acid at the expense of water, and such displacement will be greater the greater the concentration of acid. Similar considerations apply to colour acids, and in both cases the reduction of plasticity will increase with the amount of acid combined. This is exactly in accordance with experiment.

In presence of alkali, on the other hand, the whole tendency will be towards increased plasticity, on account of the following series of reactions :—

$$R_1{-}CO{-}NH{-}R_2 + NaOH \longrightarrow R_1{-}C(ONa) = N{-}R_2$$
$$+ H_2O \longrightarrow R_1COONa + R_2NH_2.$$

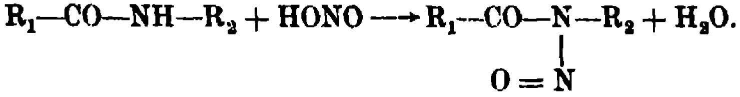
When all the alkali is removed by washing, the two amino acids are free to re-combine, and no permanent change need occur if degradation is confined to the polymerisation linkages.

The action of formaldehyde in reducing the plasticity of wool is dual in character. The molecular structure is strengthened by the formation of condensation products between contiguous —CO—NH— groups, and at the same time the affinity for water is reduced, preventing the formation of successive degradation products. The reaction may be represented as follows :



$$2\,R_1{-}CO{-}NH{-}R_2 + CH_2O \longrightarrow \begin{array}{c} R_1{-}CO{-}N{-}R_2 \\ | \\ CH_2 \\ | \\ R_1{-}CO{-}N{-}R_2 \end{array} + H_2O.$$

The reduced plasticity of "diazotised wool" appears to be due to the formation of nitroso compounds having a reduced affinity for water :



$$R_1{-}CO{-}NH{-}R_2 + HONO \longrightarrow \begin{array}{c} R_1{-}CO{-}N{-}R_2 \\ | \\ O = N \end{array} + H_2O.$$

Free amino groups are, of course, reactive with all the above reagents which combine with imido groups. It is, however, difficult to see what part reactions with amino groups can play in preventing plasticity. The free amino groups in wool must have a greater affinity for water than the imido groups, and will combine with water at lower humidities. It is significant that plasticity is developed only at high humidities. Adsorption of water by amino groups produces a weakening of the fibre by reducing the field of force surrounding each group, but combination with acid instead of water should produce a similar weakening.

Similarly, when wool is treated with nitrous acid, amino groups are replaced by hydroxyl groups, but such replacement can hardly increase the attraction between molecules. The introduction of methylene groups into wool by the action of formaldehyde on amino groups is still less likely to reduce plasticity. While, therefore, such reactions with amino groups may reduce the hygroscopic capacity of wool, it is highly improbable that they are effective in reducing the fibrillar plasticity of wool in water

Summary.

1. The elastic properties of wool are those of a structure consisting of elastic and plastic elements arranged in parallel.

2. Wool fibres in water are imperfectly elastic owing to the plasticity and rupture of fibrillæ within the constituent cells.

3. The fibrillar plasticity of wool in water is due to hydrolytic changes associated with the peptide linkages.

4. The plasticity of wool can be reduced by those reagents which react or combine with imido groups, reducing their affinity for water and inhibiting hydrolysis of the peptide linkages.

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Artefacts as a Guide to the Chemistry of the Cell.

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[PLATES 9 and 10]

At the end of the account of some observations offering a possible explanation of the appearances known as "Golgi bodies and apparatus" in fixed material, which were published last year ('Roy. Soc. Proc.,' B, vol. 101 (1927)), I suggested that a way might thereby be opened to the investigation of certain processes of metabolism within the cell. The difficulties in the way of making any further step were considerable. As was then pointed out, not only does the lipin content of the cells of different tissues vary greatly, but it varies also in similar cells under different physiological and pathological conditions. Now, as then, I refer mainly to lecithin and kephalin. Moreover, the method described in the paper referred to depends upon the degree of unsaturation of the fatty acids of the lipins, and this again is highly variable, even in similar cells, under different physiological conditions.

If the interpretation of the observations given last year be correct, then the variations in the appearances in fixed material known as "Golgi bodies" is amply accounted for by these variations in the lipin content of the cells both as regards quantity and degree of unsaturation.

Some attempts were made to make standardised preparations of lecithin and kephalin, and to compare the effects produced by the osmic acid method of demonstrating "Golgi bodies" upon mixtures containing them, with similar appearances produced by similar methods in the cells of various tissues. To make such comparisons of any value it would have been necessary to produce at least the lecithin and kephalin used in the mixtures, of a definite degree of purity and unsaturation. Owing partly to the limited means at my disposal and partly to the inadequacy of available knowledge with regard to the lipins, and to the difficulty of preventing them becoming saturated or oxidised during the process of isolation, these attempts were unsuccessful.

The line of investigation eventually followed was suggested by the great changes described as occurring in the "Golgi bodies" and apparatus as the result of different diseases and injuries. Modifications in these appearances are described in the cells of cancer (Veratti, 1909; Savaguone, 1910; Tello, 1913; and others); as following the section or injury of nerves (Martinotti, 1904; Marcora, 1910; Ramón y Cajal, 1915; Penfield, 1921, and others), as being produced in the cells of certain glands in animals suffering from phosphorus poisoning (E. V. Cowdray, 1924), and in many other conditions.*

The difficulties in the way of this particular investigation are no less, but sometimes greater in most of these cases, than in the case of normal cells; but phosphorus poisoning seemed to offer an opportunity not given by the others. Here only was a definite chemical cause of difference between the cells, which might possibly be reproduced artificially in the mixtures. In the other cases there exists little or no suggestion as to what the chemical changes in the cell may be, and none at all as to the possibility of reproducing them. As the method of making standard mixtures for the purposes of comparison was to me impracticable, it seemed that the next best thing to do would be to try to reproduce in the mixtures, by empirical experiments, modifications known to follow phosphorus poisoning in the cells of the organs of the animal.

E. V. Cowdray (1923, 'Science,' vol. 58, pp. 1–7, and 'General Cytology,' 1924) describes changes which occur in the Golgi apparatus of the cells of the pancreas in the guinea-pig as the result of phosphorus poisoning. (Plate 9, see figs. 1 to 6.)

These changes are quite striking and distinctive. They are described by Cowdray as "disintegration (of the 'Golgi apparatus') but no migration of fragments into peripheral cytoplasm." His figures are very suggestive. They show a decrease in the black (osmicated) "Golgi apparatus" which changes from a thick anastomosing net near the nucleus, into a few little black specks. As this change in the black staining material goes on, it seems to me that there is an increase in definition and also in volume of a number of what appear in the figures to be vacuoles. These vacuoles seem to coalesce in the later stages, and to be clearly defined, remaining perfectly free of all darkening after prolonged treatment with osmic acid. I have repeated these experiments of Cowdray's and obtained similar results.

If the Golgi bodies and apparatus be, as I believe, simply the lipin content of the cells separated out from the rest of the constituents by the methods used in fixation, then these appearances produced in the cells of the animal by phosphorus poisoning suggest very strongly that one of the effects is to saturate the fatty acids, at any rate in the cells examined, for if the fatty acids be saturated or oxidised the lipins and fats will not blacken when treated with osmic acid These structures which have the appearance of vacuoles I take to be simply globules of lipin or fat of which the fatty acids are saturated While poisoning is produced only by yellow phosphorus, and not by phosphorus in any other form whether in combination or not it did not seem probable that the changes in the individual cells could be due to the direct action of yellow phosphorus but to some combination formed in the body and carried to the cells A number of substances were tried

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* All quoted in "Pathology of the Golgi Apparatus." Cowdray, 'General Cytology,' pp. 347–9. Chicago University Press (1924).

Various amounts of sodium hypophosphite and other compounds of phosphorus were added to those mixtures which produce appearances similar to the Golgi apparatus when treated in a suitable manner (*Op cit supra*) These mixtures were kept at a temperature of 30° C for varying periods and then films were made suitably fixed and treated with osmic acid None of these experiments produced any striking results The addition of sodium hypophosphite produced a certain amount of saturation of the fatty acids but nothing approaching the drastic changes evident in the cells examined

Eventually I found that yellow phosphorus is freely soluble in methyl myristate and methyl laurate, which are what I used to form the artificial nuclei in my mixtures Instead of using these in a pure form in making the temporary emulsions to add to the mixtures I used myristate and laurate in which phosphorus had been dissolved The results were very striking when the mixtures with and without phosphorus were compared

With the particular mixtures used * when no phosphorus was present the lipins took the form of coiled up threads loops and small masses which blackened with osmic acid (figs 7 and 8) There were some cases in which the lipins appeared to have penetrated the globules of myristate or laurate (fig 9 Plate 10) This is the same result as was described last year

When myristate or laurate in which phosphorus had been dissolved was used and the mixture kept at a temperature of 30° C for two hours the appearance after fixation and treatment with osmic acid was quite different A considerable proportion of the lipins appeared to be collected upon the surface of the globules in small masses joined by a network The structure of both the masses and network was granular in appearance (Plate 10, see figs 10 and 11) The phosphorus is more freely soluble in the laurate than in the myristate, and the appearances were more striking when the former than when the latter was used in the mixture, but in both cases about 80 per cent. of the globules examined showed the material blackened by the osmic acid distributed upon their surfaces in the manner described, whereas this position was never taken by the lipins in the control films made at the same time with the same mixture without the phosphorus dissolved in the myristate or laurate.

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* Gelatin, 2 per cent egg white, 5 per cent lecithin (commercial B D H), 0 3 per cent , sodium chloride 0 6 per cent peptone, 0 5 per cent , methyl myristate or laurate, 0 5 per cent

A great point is made by some observers of the constant position of the "Golgi apparatus" in certain cells in relation to the nucleus It would seem, judging by the observations just described, that this is not incompatible with the interpretation of the "Golgi apparatus" as being the lipin content of the cell separated out by the methods used in fixing the material, for if the presence of phosphorus in the globules of myristate and laurate can determine the relative position of the separated lipins and globules, there may well be many conditions of the nucleus which would produce a similar effect

If the mixtures are kept at a temperature of 30° C for a longer period, the fatty acids are apparently gradually oxidised Films made after 24 hours at 30° C show little or no material that blackens under treatment with osmic acid after fixation Instead we find groups of structures having the appearance of vacuoles, frequently adjacent to the globules of myristate or laurate In some cases there are a few black granules among them The appearance is generally similar to that found in the cells in the later stages of phosphorus poisoning (figs 13 to 16)

There were certain appearances in the mixtures kept at 30° C which suggested that they might have been infected with some micro organism, and that the changes described might be due, in part at any rate, to the action of these and not to the interaction of the known contents The experiments were repeated, with special precautions at each stage, to ensure that the mixtures were sterile and culture tubes were inoculated from each at the same time that films were made Only those films have been described which were shown to have been made from sterile mixtures

After about 40 hours at 30° C changes took place in the mixtures which were unlike anything described as occurring in cells After 4 days the appearance presented by the fixed and osmicated film was that of globules of myristate or laurate in a practically homogeneous matrix The matrix was dark in the case of the mixtures without phosphorus, and pale yellow in the mixtures which contained myristate or laurate in which phosphorus had been dissolved.

While I have no doubt as to the accumulation of the lipins on the surface of the globules which contain phosphorus as described above, and the absence of this phenomenon in the same mixture in which the phosphorus is lacking, it is necessary to point out that a somewhat similar accumulation has occurred in other films with which I have experimented Last year I described and figured a similar appearance (*op cit supra*) In fig 12 a globule with the lipins distributed on the surface is shown but in these cases I have been unable to ascertain what circumstances determine the position of so large a proportion of the lipins It appears to occur in only a few globules in any film and to be more likely to happen when kephalin is the preponderating lipin The appearance is not quite like that produced by the presence of phosphorus, where such a large proportion of the globules have the lipins distributed on their surfaces

The facts ascertained seem to be that the presence of yellow phosphorus in solution in the myristate or laurate causes the lipins in this particular mixture to accumulate on the surface of the globules when a film is made and fixed after a period of about two hours at a temperature of 30° C while where no phosphorus is present the lipins are collected elsewhere A further period at 30° C causes saturation of the fatty acids of the lipins and also appears to cause them to collect in globules on fixation In view of these observations it seems quite possible that the behaviour of the lipins in fixed preparations of cells may be due to a reaction produced by something in the nucleus of the living cell

It is certain that chemical as well as physical changes are produced in most of the constituents of the cell by fixation and that these changes will vary according to the fixative used Hence it is difficult to estimate the value of any appearance produced in microscopic specimens by treatment applied after fixation is complete In the case of these experiments however the position of the lipins in relation to the globules appears to be determined by the action of the fixative on the mixture and varies in one case at any rate, with the presence or absence of a substance in the globule which reacts on the surrounding medium These experiments suggest also that a change in the behaviour of the lipin content of the cell may be produced by a reaction from within the nucleus

As has already been stated the lipins appear to be collected on the surfaces of about 80 per cent of the globules after fixation if the mixtures containing myristate in which phosphorus has been dissolved are used In the absence of phosphorus this happens only in the case of a few of the globules and then the arrangement of the lipins differs considerably When the phosphorus is absent the overwhelming proportion of the lipins in fixed preparations is found in the neighbourhood but not on the surface of the globules, but the results have varied in so erratic a manner in different films prepared from similar mixtures and treated in apparently the same way, that I am not in a position to suggest any other causes controlling these differences or the proportionate numbers of different distinguishable appearances though such causes must exist

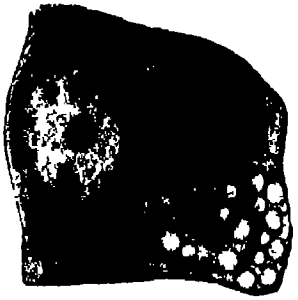
The method suggested by these experiments of investigating the nature of the chemical and physical changes which undoubtedly occur in individual cells in various physiological and pathological conditions differs from those used by Fischer Hardy Butschli Gustav Mann and others Their investigations have explained much with regard to the physical appearances produced by fixation and with regard to the micro chemistry of various methods of staining All this however is of a different nature to the method I suggest We are dealing with a number of variable and unknown factors and the best we can claim for what is found in fixed material, both before and after staining is that the appearances have been produced by the action of certain reagents upon cells Constant differences in such appearances in similar cells after similar treatment may be attributed to variations of physiological state or to definite pathological conditions

So far however no information is thus obtained as to the differences of physiological state or as to the changes due to pathological condition But now it is suggested that by applying to films or drops of experimental mixtures of materials representing possible chemical cell contents the same methods of fixation and staining as are used in dealing with the cells themselves similar microscopic appearances found in the two different cases may help to identify the nature of the new chemical condition which is responsible for this appearance within the cells Thus the addition of phosphorus to the globules representing nuclei has reproduced two changes in appearance that occur in cells and it may be that by varying the mixtures and obtaining other reproductions of appearances occurring in cells under certain conditions some evidence as to the chemical changes and how they occur in individual cells in different physiological and pathological conditions may be secured

Conclusions

When a cell is fixed the position and arrangement of the lipins in relation to the nucleus are probably due to or are influenced by some chemical change in the nucleus

When methyl myristate or methyl laurate in which yellow phosphorus has been dissolved are added in the form of an emulsion to certain colloidal mixtures



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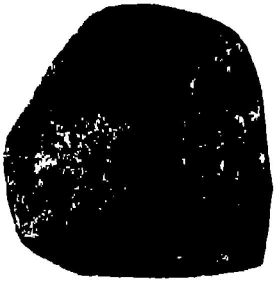

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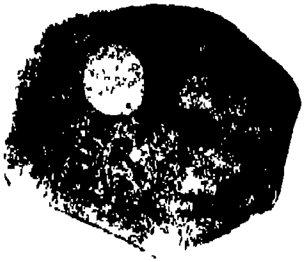

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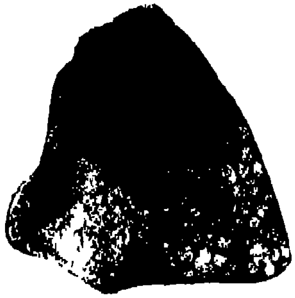

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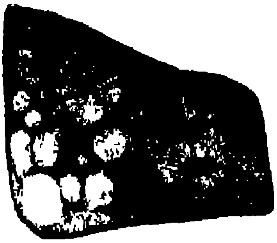

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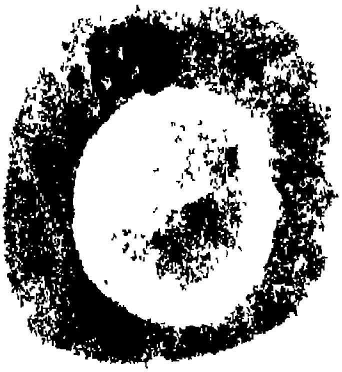

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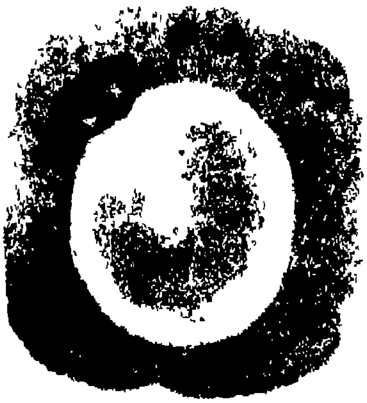

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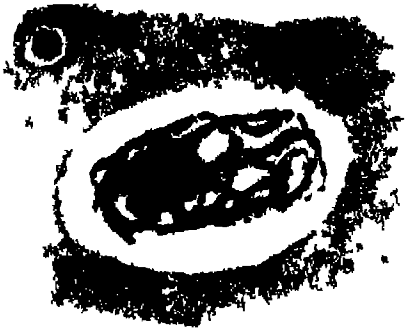

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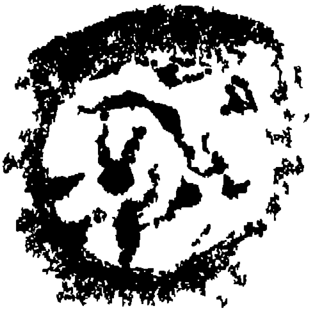

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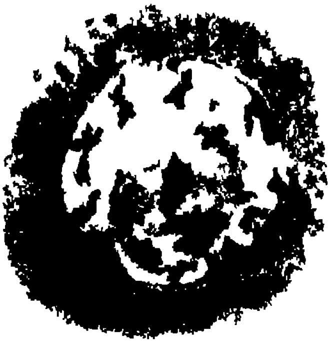

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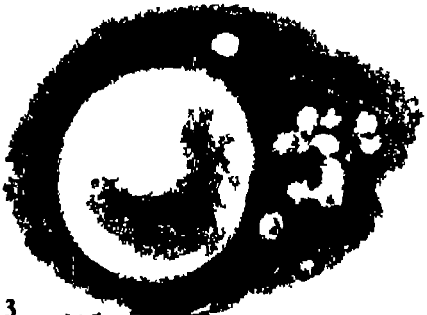

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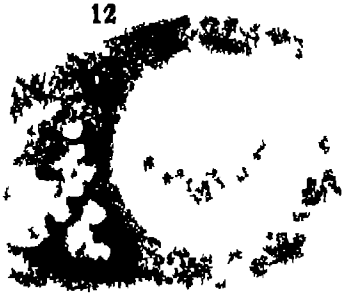

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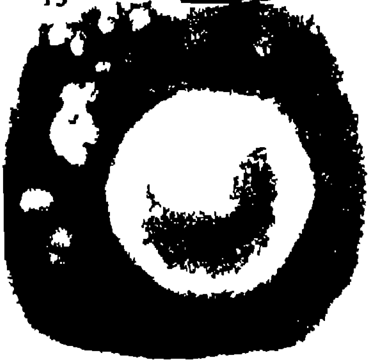

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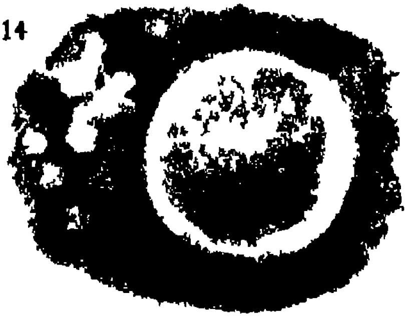

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and kept at a temperature of 30° C the microscopic appearances presented on fixation and treatment with osmic acid show that in about two hours a large proportion of the lipins are distributed over the globules while after about 24 hours most of the fatty acids of the lipins have become saturated or oxidised

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DESCRIPTION OF PLATES

Plate 9

Figs 1-6 —(Copied from General Cytology Chicago University Press 1924 by permission of Dr E V Cowdray Illustrating the effect of phosphorus poisoning) Cells from the pancreas of the guinea pig prepared by Kopsch s method Blackening of the Golgi apparatus and its progressive disintegration and final disappearance

Fig 7.—Film Gelatine 2 per cent egg white 5 per cent lecithin (commercial B D H) 0 3 per cent sodium chloride 0 6 per cent Witte s peptone 0 5 per cent methyl laurate in temporary emulsion 0 5 per cent in water Film fixed with Mann s fluid Osmic acid 2 per cent 4 days

Fig 8 —Film As fig 7

Plate 10

Fig 9 —Film As figs 7 and 8

Fig 10 —Film As fig 9 but with yellow phosphorus dissolved in methyl laurate and mixture kept for 2 hours at 30° C before film was made

Fig 11 —Film As fig 10

Fig 12 —Film Egg white 7 25 per cent gelatine 1 per cent peptone 0 5 per cent kephalin 0 2 per cent (prepared as described in Roy Soc Proc B vol 101 1927 p. 481) in water Mann OsO_4 2 per cent 4 days

Fig 13 —Film As fig 10 but mixture kept for about 20 hours at 30° C before making film

Fig 14 —Film As fig 12 but methyl myristate used instead of laurate

Fig 15 —Film As fig 12 but kept at 30 C for 30 hours

Fig 16 —Film As fig 12 but kept at 30 C for 36 hours

Note —A saturated solution of phosphorus was made in the methyl myristate and laurate, and this was diluted to 1 in 8

Antiseptic Compounds Some Further Derivatives of Anilquinoline *

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(Received June 8, 1928)

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BIOLOGICAL SECTION

It was shown in a former paper (Roy Soc Proc ,' B vol 96, p 317) that a number of derivatives of amino anilquinoline acted as powerful antiseptics, being especially potent against *B coli* The present communication forms a continuation of this work, and describes further compounds of the same general type †

Antiseptic power was estimated as described in the previous paper (*loc cit*) The results there recorded pointed to a tendency towards increased antiseptic action as the mass of the molecule was augmented provided that the " alternate linkage system " was preserved intact

In the anil series the 6 methylquinoline compound (48) was more active than the unsubstituted anil (47) and the β naphthoquinoline derivative (52) was still more potent Again the higher acylamino compounds (65–69), as well as the acetylamino derivative itself (59) were very powerful antiseptics The same tendency is also apparent when the unsubstituted amino anil (41) is compared with the corresponding 6 methyl derivative (42)

It appeared of interest therefore to prepare further compounds of larger molecular mass

Variation of the Quinoline Nucleus

Little could be done in the direction of increase of mass of the quinoline nucleus owing to limitations of solubility The comparatively low activity of some of the substances prepared *e g*, No 98 may be partly due to this cause The urethane derivatives (95 96 97) may be regarded as developments of the acetylamino quinoline compounds previously described (58–69), and they

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* The work reported in this communication was done with the support of the Medical Research Council

† The numbers and tables follow those previously published

exhibit the very powerful antiseptic action characteristic of this group There is practically no difference between the potency of the methyl and ethyl esters As will be observed quinaldylurethanes have produced exceedingly active substances when condensed with other nitroso compounds (111 114 117)

The phenyl uramido compound (98) showed only a weak action but its solubility is low The acetylamino β naphthoquinoline compound (99) although rather insoluble has a fairly powerful action on *Staphylococcus aureus* outside the zone of precipitation but differs from most of the anils in its comparatively weak action on *B coli* whereas the corresponding unsubstituted β naphthoquinoline compound (52) is very active

Variation of the Benzene Nucleus

The solubility factor also interferes to some extent with efforts to increase the mass of the benzene portion of the molecule but in certain cases very powerfully antiseptic compounds have been obtained of a satisfactory degree of solubility Various nitroso compounds have been condensed with quaternary salts of 6 methylquinaldine β naphthoquinaldine 6 acetylamino quinaldine and ethyl quinaldyl carbamate these being the intermediates which gave rise to the most potent substances in the dimethylamino anil series The particular quinaldine derivative selected was determined in some cases by the solubility of the final product

The addition of further aromatic nuclei to the benzene nucleus does not appear to be effective since the compound derived from nitrosoethylbenzylaniline (100) was relatively weak against *B coli* in serum and that prepared from *p* nitroso diphenylamine (101) was inactive throughout (compare with 116) Similarly, the condensation products of nitrosodimethyl and diethyl naphthylamines had only slight action (102 103 104) The di anils derived from dinitroso diphenylpiperazine (105 106) were weak especially against *B coli*

The *cyclo* hexyl compounds (107 108) however where the additional nucleus is reduced and assumes an aliphatic nature were considerably more active as shown by comparison of 108 with the corresponding phenyl compound (101) Very potent substances were obtained from *p* nitroso tetrahydro quinoline and *p* nitroso methyl tetrahydro quinoline (109 114) where the additional nucleus is again reduced In view of these observations it is proposed further to examine the higher alkylated derivatives of the amino anilquinolines

Nature of the Basic Group in the Benzene Nucleus.

It was noted in the previous paper that the substitution of a tertiary for a primary basic group in the benzene nucleus increases the antiseptic action (compare Tables IV and V). On comparing compounds prepared from nitrosotetrahydroquinoline, in which the basic group is *secondary*, with similar derivatives from nitrosomethyltetrahydroquinoline, where the basic group is tertiary, it is seen that there is no significant difference in activity. For further comparison, the corresponding monomethylamino anils were prepared, and were found to be as potent as the dimethylamino compounds (compare 115, 52; 116, 59; 117, 97). Thus it is evident that in this group of compounds the distinction, as regards antiseptic potency, lies between those containing, on the one hand, a primary basic group, and on the other, a secondary or tertiary group, in the benzene nucleus.

CHEMICAL SECTION.

The anilquinoline compounds were all prepared by the general method of condensing the appropriate nitroso compound with the quaternary salt of the quinaldine derivative, in alcoholic or aqueous-alcoholic solution, as described in a former paper (' Journ. Path. and Bact.,' vol. 27, pp. 121–22 (1924)). In most cases a small quantity of piperidine was used as condensing agent, but the nitroso compounds of dimethyl- and diethyl-α-naphthylamine condensed readily without the addition of piperidine. In some instances (100, 103, 109, 112, 115) better yields were obtained by condensation with the methiodide of the quinaldine derivative, the methochloride or methoacetate being subsequently prepared by boiling in alcoholic solution with the appropriate silver salt. In preparing the phenyluramido compound (98), the metho-acetate was condensed, the product crystallising well from the alcoholic solution.

Like the anils previously described, the products were obtained in well-formed crystals, usually prisms or needles, showing a blue or green reflex, and gave blue or violet solutions in alcohol, the aqueous solutions being somewhat redder in appearance.

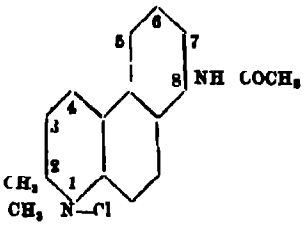
Intermediate Compounds.

The preparation of some of the intermediate compounds has already been described (' Roy. Soc. Proc.,' B, vol. 96, p. 317). The following may be mentioned in addition :—

Methyl and ethyl quinaldyl carbamates were made by the action of methyl and ethyl chloroformates on 6-aminoquinaldine in chloroform solution The mixture was warmed on the water-bath for one hour, a yellow crystalline precipitate of the hydrochloride of the carbamate being formed The base was obtained by the addition of ammonia, and crystallised from dilute methyl alcohol in colourless prisms or needles The methyl ester melted at 182–3°, and the ethyl ester at 150 5° The former could be made satisfactorily by leaving the mixture for several hours at room temperature, but heating was necessary with ethyl chloroformate

Both substances were converted to their methochlorides in the same way as 6-acetylaminoquinaldine, described in the former paper

8 *acetylamino* β *naphthoquinaldine methochloride*



5 nitro β naphthylamine was first prepared by the method described in D R P 57491 (Friedlander III 508) The mixture was kept at 0° to 5° during nitration Several recrystallisations were necessary in order to obtain the pure 5 isomer The 5 nitro β naphthylamine was condensed with paraldehyde in presence of hydrochloric acid by the ordinary Dobner Miller method The mixture was heated on the water bath under a long reflux condenser for four hours, with frequent shaking The pale yellow solid first formed gradually dissolved, some dark resinous matter being produced The mixture was poured into water and the solid filtered pressed, and extracted with hot glacial acetic acid with the addition of charcoal From the cooled and filtered solution the hydrochloride of the quinaldine base separated

The base itself was obtained by the addition of ammonia and was recrystallised from alcohol It forms almost colourless plates melting at 166–7° The yield was low, being 20 per cent of the theory The nitro compound was reduced with stannous chloride and hydrochloric acid the resulting amino derivative being crystallised from dilute alcohol It forms minute crystals, melting at 169–70° The acetyl compound, prepared by acetylation with acetic anhydride and fused sodium acetate, was crystallised from dilute alcohol and was obtained in colourless needles of melting point 235–37°

0 2075 grm gave 21 3 c c N at 21° and 750 mm
N = 11 60 per cent
$C_{16}H_{14}ON_2$ required N — 11 21 per cent

The methochloride was obtained in the same way as the corresponding quinaldine derivative Methyl *p* toluenesulphonate could be used instead of dimethyl sulphate for methylation and the methochloride readily separated on the addition of brine to the aqueous solution of either the methosulphate or the metho *p* toluenesulphonate

6 *phenyl uramidoquinaldine* was prepared by the action of phenyl *iso* cyanate on 6 aminoquinaldine in chloroform solution at 20–25° The product separated on standing and was obtained in the form of fine needles by re crystallisation from nitrobenzene It decomposes without melting above 220° The methosulphate was made in the usual way by the action of dimethyl sulphate in nitrobenzene solution and by addition of sodium acetate to the aqueous solution of the methosulphate the metho acetate was obtained and, after filtration and pressing was used direct for condensation with *p* nitroso dimethylaniline

Nitroso Compounds

The nitroso derivatives of the secondary bases were made in the usual way from the nitrosamines by the action of hydrogen chloride in alcohol or alcohol ether solution

p nitroso *cyclo* hexylaniline crystallises from ether in large blue green prisms melting at 91 93°

The nitroso compounds of the tertiary bases were obtained directly by the action of nitrous acid the method of Friedlander and Welmans (Ber vol 21 p 3125 (1888)) being used in the case of the derivatives of dimethyl and diethyl α naphthylamines

In the following tables—

The figures representing antiseptic potencies are the reciprocals divided by 1000 of the dilutions which produce the effects indicated In the columns headed *precipitation* the figures similarly converted record the highest dilution within the range investigated at which precipitation occurs in peptone water (P) and serum (S) respectively the sign — indicates absence of precipitation

Table VII.—Dimethylamino anils with Variation in the Quinoline Nucleus

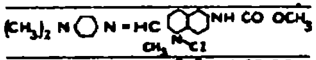
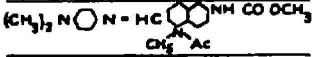
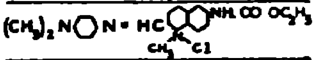
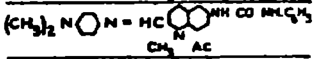
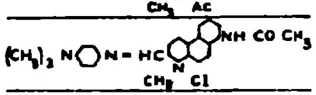
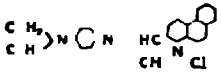
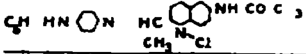
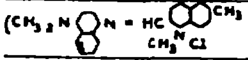
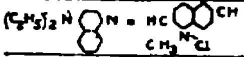
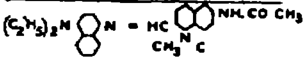
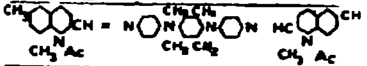
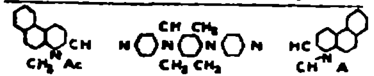
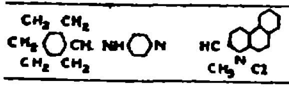
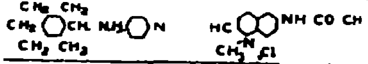
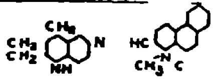
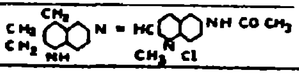
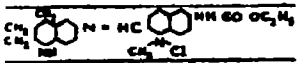
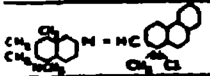
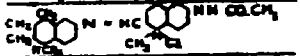
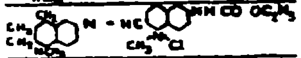
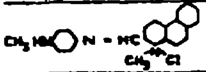
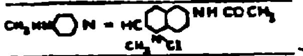
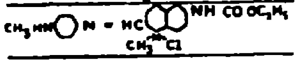
No	Substance	*Staphylococcus aureus*						*B coli*						Precipitation.	
		Peptone water			Serum			Peptone water			Serum			P	S.
		+		−	+	+	−	+	±	−	+	±	−		
95		?		1000	?		1000	400		200	?	1000	400	—	—
96		?		1000	?		1000	1000		400	?		1000	10	—
97		?	2000	1000	?		1000	1000		400	4000	2000	400	—	—
98		20	10	4	200	100	40	100	40	2	400	200	10	100	10
99		1000		400	400	200	100	200	100	4	200	100	20	10	1

Table VIII —Variation in the Benzene Nucleus

No	Substance	*Staphylococcus aureus*						*B coli*						Precipitation	
		Peptone water			Serum			Peptone water			Serum				
		+	±	−	+	±	−	+	±	−	+	±	−	P	S
100		?		2000	1000	400	200	2000	1000	200	400	200	40	100	—
101		2		?	4	2	?	2		?	10		4	40	—
102		400	200	100	40	20	4	100		40	40	20	10	10	—
103		200		100	40	20	4	40	20	10	10	4	2	10	—
104		100		40	40	20	4	2		?	4	2	?	10	—
105		200	100	20	200	100	20	40	20	2	4	2	?	4	2
106		1000	400	100	400	200	40	20	10	1	20	10	4	10	—
107		?	1000	400	200	100	10	200		100	100		40	10	—
108		1000		400	100	40	10	200		100	100	40	20	10	—
109		10000	4000	2000	4000	2000	1000	?	1000	200	2000		1000	—	—
110		?	1000	400	1000	400	200	400		200	1000		400	4	—

111			10000	4000	2000	•	4000	1000	4000		2000		4000	400	10	—
112			10000	4000	1000	1000	400	200	2000		1000	200		100	—	—
113			4000	2000	1000	2000	1000	400	2000	1000	200	4000	2000	400	—	—
114			4000	2000	1000	400	200	100	10000	4000	2000	2000	1000	400	—	—
115			10000	4000	2000	2000		1000	2000	1000	400	1000	400	200	—	—
116			4000	2000	400	2000	1000	400	1000	400	200	2000	1000	400	—	—
117			4000	2000	1000	4000	2000	400	1000		400	4000		2000	—	—

Analytic Studies in Plant Respiration. I.—*The Respiration of a Population of Senescent Ripening Apples.*

By F. F. Blackman, F.R.S., and P. Parija, Ravenshaw College, Cuttack, India, formerly Frank Smart Student in the University of Cambridge.[1]

(Received July 4, 1928.)

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Introduction.

Of all protoplasmic functions, the one which is, by tradition, most closely linked with our conception of vitality is the function for which the name of respiration has been accepted. It might, therefore, well be expected that every variation of the intensity of the metabolic activity of a cell would be correlated with some change in the respiration of that cell. Before we can decide whether respiration really holds this position as an index of the integrated activities of the cell, we need to accumulate respiration data for different types of plant organs throughout their life-history of development, maturity and senescence. These data must then be examined critically with the hope of establishing the nature of the major and minor determinants of the variations of intensity of respiration. No such collection of data has yet been published. The present paper aims at making a contribution to this collection and other contributions should follow.

A good deal of work has already been carried out in the Cambridge Botany School upon respiration of evergreen leaves, which continue to exist in a state of maturity for very long periods of time. In striking contrast with this type of organ is the type of the ripening fruit. The natural biology of the two is so different that it is obviously of importance to establish whether the same fundamental principles are manifest in both. In the ripening fleshy fruit, senescence is the dominant stage of ontogeny. The fruit of the apple, which possesses such striking keeping properties, is most suitable for investigation, since it runs through its ripening senescence at a slow rate.

An opportunity of taking up such work was provided by the beginning of the experimental work of the Food Investigation Board, at Cambridge, under Sir William Hardy. The apples made available for us were those that were kept in cool storage at about 2·5° C. for the investigations of Dr. F. Kidd and Dr. C. West. We are indebted to the Board for a subsidy to enable the junior author to devote a year to the investigation presented in this and the following papers.

The Outlook and the Problems.—Our outlook upon these apples has been to regard them as a population of individuals slowly progressing in cool storage through the metabolic drift which constitutes the senescent and penultimate stage of ontogeny, popularly spoken of as ripening. Some biological truths of this drift can be brought out by statistical treatment of the population as a whole, others only by intensive study of individual behaviour. It is the latter aspect that we wished to explore, in order that we might find out what features of individual respiration can be held to be capable of physiological interpretation and which must, at present, be regarded as indeterminable chance happenings.

The nature of the test applied to the respiration of the apple population was to take out of store, throughout a period of eight months, individual apples, one by one, and examine the intensity and course of their respiration in air, and also, as part of the same enquiry, to subject them to a variety of oxygen mixtures ranging from zero to 100 per cent. The survey of the results in air are given in the present paper, while those in the oxygen mixtures are brought together in the following papers.

The previous history of the population was that they were Bramley's Seedling apples grown on fen soil, gathered at the beginning of October, 1920, and maintained in cool storage between 2° and 2·5° C for the investigations of Dr. Kidd and Dr. West, to whom our thanks are due for this essential assistance. The apples were picked from one orchard at one time and believed to be a homogeneous population, though they had not been gathered or graded under scientific supervision. When once brought into store the population is, of course, exposed to an extraordinary and quite unnatural uniformity of environment – no change of temperature or humidity, and no alternation of light and dark, for months in succession. One interest of our investigation would be to find out how far the population declared itself homogeneous under our physiological tests.

The individual apples were brought to the laboratory under standardised conditions and investigated at one temperature only, namely, 22° C. No conscious selection was exercised in taking individuals from store, except the avoidance of any that were bruised or showed traces of brownness. The average condition of the population was, of course, changing with the progress of the metabolic drift and this revealed itself by the gradual colour change from full green through yellow-green to golden yellow and finally brown.

Allowing for all this drift, the conclusion was yet forced upon us by the results of our work that the population could not be described as homogeneous. Clearly, the apples continued to be distinguishable by physiological characteristics that differentiated them on the day that they were picked and put into store. The fact that recent differences of environment were negligible as a contributory factor to the observed differences of behaviour encouraged us to persevere in the endeavour to explain observed differences in terms of initial inherent qualities and temporal physiological drift.

Our observations of their respiration at 22° C. were continuous and revealed many minutiæ of difference in behaviour, all of which we have endeavoured to bring to account, and either interpret them or formulate problems as to their determination. It is at least made clear, that had three or four apples instead of one been employed in each experiment, yielding merely average results of behaviour, then it would not have been possible to push our analysis very far. It is an essential consequence of the metabolic drift in storage that results obtained in one month are not repeated exactly in the next. When any problem arises in this type of work, it is not possible to go back and repeat an observation on identical material once more.

Experimental Methods and Procedure.—The apples were brought from the cool store to the laboratory, weighed, and placed singly in a glass respiration-chamber of a spherical form, which consisted of two hemispherical domes with a wide equatorial flange and two polar tubes as inlet and outlet for the constant current of gas, maintained by aspirators through the chambers, at a rate of about 1500 c.c. per hour. The chamber halves were waxed together, fixed in a weighted frame and lowered into a large thermostat bath kept at 22° C. The whole of this occupied about 30 minutes; the air currents were then started at their proper rate and run for about 90 minutes as a preliminary before estimations were started. There is therefore about 2 hours of respiration in all before the point of time that figures as zero time in the records. The bath temperature generally kept constant within 0·2° C.: there were a few misadventures during the nine months' work, which are noted in the records.

of the individual cases. The current of air passes from the chambers through Pettenkofer tubes of standardised baryta, which are arranged as a parallel set, and the current is shifted on automatically by clockwork from one tube to the next at intervals of three hours. In this way continuous records of the production of CO_2 can be obtained for an indefinite period of time. In the present work some records continue for 16 days without a break.

Each day the used Pettenkofer tubes are lifted out one by one, washed into a beaker and titrated with decinormal HCl and phenolphthalein. They are then refilled and replaced in their frame, ready for the air current to come round again. The CO_2 production is expressed in mgr. CO_2 per 300 grm.-hours. Medium-sized apples were selected from the store, averaging 140 gms.; they were weighed again at the end of the experiment; the loss of weight averaged 1·5 per cent. per 10 days, the minimum being 0·77 per cent. and the maximum 2·09 per cent. The individual cases will be found detailed in the Appendix to the next paper.

The Respiration Records.—In all, 21 experiments, numbered V to XXV, were carried out, in that sequence, on single apples brought from cool store, from the middle of November, 1920, to the end of June, 1921. The CO_2 production of some was examined in air only, but most were exposed as well to the effects of one or other of the following concentrations of oxygen:—Zero per cent. (nitrogen), 3 per cent., 5 per cent., 7 per cent., 9 per cent., and 100 per cent. O_2.

The work involved nearly 2000 estimates of the CO_2 of respiration: the numbers are not tabulated in this paper but presented in graphic form throughout. The graphic records of the respiration values of the 21 experiments will be found set out in the Appendix to the next paper; these records will be referred to as the "General Charts" of the results. Mostly two experiments were carried on concurrently; and where this was so, the two records are grouped together in the same chart, one often serving as control to the other.

In the various sections of this paper, discussing special points, excerpts from the general charts bearing on the problem are brought together and correlated. In all the charts the ordinates express mg. CO_2 per 300 grm.-hours for the fresh weight of the apple when taken out of cool storage. The abscissæ are hours of time from the beginning of the respiration measurements. Each three-hour measurement is represented graphically by a single heavy line, or by three consecutive dots, covering the period of three hours duration.

The long series of continuous estimations show a constant tendency to fluctuate up and down. We have thought that the general drift of the respiration is brought out more clearly in our graphic records when we represent it, not by a single median line, but by two "contour lines" which are drawn parallel, one above and the other below the range of the fluctuation. When a definite numerical value is needed for respiration, it is, of course, the value midway between the contour lines that is adopted.

The Fluctuations.—It is not to be expected that under constant conditions the sequence of estimations would give values lying on one steady line, but it is clear that the fluctuations that actually occur are much greater than those that can be attributed to small random errors of titration, tube-washing and manipulation.

As a striking example of these fluctuations, it has several times been noted that, when the respiration is undoubtedly declining generally, as proved by a record lasting several days, there may yet occur in the course of it a level sequence of no less than four identical readings—covering 12 hours— before the falling drift comes into evidence again. Had the record been stopped just at the end of this 12 hours it might have been concluded, confidently, that the fall had passed into a definite level phase.

The range of the fluctuations indicated by the distance apart of the two "contour lines" is about the same in the different experiments when respiration is running an approximately level course, and amounts to 0·7 mg. CO_2, but occasionally the readings seem to swing with greater amplitude. Apple VIII provides a unique case: this was the one apple that developed a patch of fungus mycelium, involving a big rise in the respiration. Here the fluctuations became very great and the successive readings were most irregular, which we attribute to the irregular growth and activity of the fungus on the apple tissue.

The "Air-Line." When some partial pressure of oxygen, other than that of air, is given it will be seen in the various records that the CO_2 production may be either much increased or much depressed, causing a deflection of the double contour line which indicates the drift of respiration. At such times it is important to know the ratio of this increase or decrease to the magnitude of respiration that would have occurred had the apple been kept in air all the time. For this purpose it is necessary to join together the air records before and after by an interpolation. This is represented in the records as a single median line, and not by contour lines. Often these interpolations have to be long, and much study of the records has been required to carry them out with confidence.

Joining up the actual air records by interpolations, and sometimes adding extrapolations, we can get a continuous line, which we shall call the "air-line," running right through the experiment and suitable for comparisons and controls.

Respiration in Air. the Special Problems.—We may conclude this introduction by indicating the chief features and problems presented by the respiration of our 21 apples, when we came to review the records obtained. The earliest apples, V and VI, were investigated when they had been in store for only 30 days; the latest, XXIV and XXV, when they had been stored for 260 days.

(*a*) The primary variable was the absolute magnitude of the respiration for different apples. Some air-lines started as high as 20 mgr. CO_2 per 300 grm.-hours apple while others were as low as 12 mgr. CO_2. To some extent the drift of these initial magnitudes was temporal, but clearly some other quite different factor was involved as well. This complication is to be examined in the first section.

(*b*) Apart from differences of pitch the air-line records were not all of the same type. Some declined fairly fast, some kept level for a time and then fell, while others rose day after day. The outstanding complication was that these different forms did not present themselves as one uniform drift of type, as the individuals were examined month after month. The resolution of these complications in the form of the drift of the air values is the subject of the second and third sections.

(*c*) A minor feature that attracted attention was the form of the air-line initially, immediately after heating up from 2·5° C. to 22° C. The rising respiration did not simply mount up to the air-line value but in many cases clearly overshot that value and then fell back to it. This special disturbance of initial rates is examined in Sections IV and V as the "change of temperature effect."

Section I.—The Intensity of Respiration of Apples during the Senescent Phase.

The problem that we have to take up in this section is that of the great variation of intensity of respiration shown by apples removed at intervals from the cool store over a period of eight months. We have 21 cases to consider, and the ideal values to work on would be the initial air-line value for each case at 22° C. Brought, as they are, from 2·5° C. the respiration rises rapidly at first but presently settles down to proceed along the air-line. Extrapolation of this air-line back to zero hour would give, for each apple, what we may call its ideal initial respiration value. There are certain complexities about the early course of observed respiration, which are to be explored in Section IV, and these affect the estimation of such initial values; but fortunately the divergences of the apples one from another are so great that for the present section it is a matter of indifference how the initials are arrived at, so long as the same method is followed for every apple. We will therefore adopt the ideal initial values just mentioned, which will be found set out in full in column 7 of the table in the Appendix to the next paper. These 21 initials are represented in fig. 1, plotted against a time axis which gives the date when each apple was removed from store and the number of days that it had existed in store since picking. Clearly the early apples give respiration values that are lowest of all, after which come slightly higher values. Subsequently there is great divergence of values, the high values mounting up to over 20 mgms. in March and falling off somewhat towards June. But all through this drift there are occasional occurrences of medium and quite low values. In the figure the highest values have been connected up with one line and the lowest with another, while a median line has been carried throughout the assembly. On this unanalysed presentation of the data one might conclude that there was

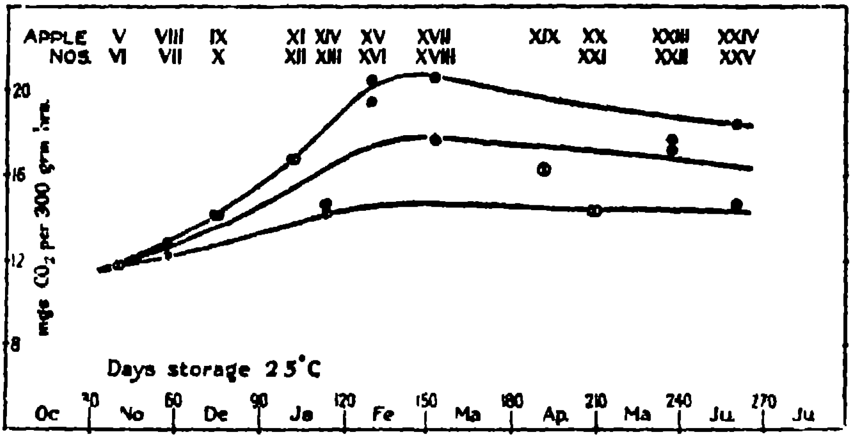

FIG. 1.—The initial respiration values at 22° C. arranged in chronological series. The time axis gives dates of removal from cool storage at 2 5°. Twenty-one apples were examined at 11 dates. The exact dates and respiration values will be found in columns 2 and 7 of the table in the Appendix to the next paper. When two apples at one date gave identical values, this is indicated by the point having a dumbbell surround instead of a circle. The serial numbers of the apples are given by the Roman numerals along the top of the figure; the top numeral refers to the apple having the higher respiration value. Lines are drawn connecting up the drift of the highest values, and the drift of the lowest values. A mean line is added equidistant from the two extreme lines.

a marked tendency for the mean value to rise till about March and then to decline somewhat to June. But more marked than the drift of the mean would be the enormous increase of the scatter about the mean with progressing ripeness. Another curious feature would be the number of examples that lie on the extreme lines and the few that are found on the median line, so that the whole assembly does not at all resemble one showing a normal scatter of chance divergences about a slowly drifting mean. Could apples really diverge so much from one another by unanalysable chance variations then nothing would be gained by working with single individual apples.

Our first progress in the analysis of this complexity came from comparing with it the grades of ripeness indicated by the colour of the individual apples at the dates when their initial respirations were determined. As the months from November to June passed there were of course changes in the appearance of the apples in store. At first, from October to March, all were full green, but then the slow progression towards ripeness caused visible change of surface colour, through yellow-green, to full yellow and then on to partial or complete brownness. While the whole population of the store was drifting through this series of changes it was clear that all individuals were not moving at anything like the same rate. In April apples representing all stages from green to brown were present. As time went on, more and more apples had to be set aside as brown in parts. Most of the apples came to ripeness (yellow colour) in April to May, but some were still yellow-green at the end of June, by which time no pure green apples were left

The correlation of colour with initial respiratory magnitude for the last seven cases first supplied a clue to the system underlying the irrational distribution of values. The earlier cases had led one to associate very low initial respiration with unripeness, and high initial respiration with ripening, but when apples XXIV and XXV were selected as the two most extreme apples for unripeness and ripeness, respectively, to be found in the store at that date, XXV being "golden yellow" and XXIV only just beyond full green, namely, "yellow green," then it was found that XXV had a low initial respiration value, 14·7, while XXIV was high, 18·5. This distinction was supported by the preceding apples, for XIX, XX and XXI, with low respiration, had been recorded as yellow, but XXII and XXIII, with higher values, as "green-yellow." Thus low initial respiration is associated with unripeness in November to December and with very full ripeness in May to June. We must conclude then that respiration first rises and then falls, and can be clearly associated with the ripening drift of colour in storage at 2·5° C.

The relation between colour and intensity of respiration (as measured here initially at 22° C.), that came out of a close study of their parallel drifts may be formulated somewhat as follows. Every apple picked unripe drifts during ripening through a special senescent phase of metabolism, the essential nature of which will be discussed in Section III. The passage into this phase from the previous phase of metabolic maturity is marked by a rise in respiration rate, which rise starts slowly, progresses faster, and then slackens off to a maximum value; during the early part of this rise the apple colour is full green, losing its intensity towards the maximum of respiration. After the maximum, the respiration begins to fall, though at first slowly, and during this stage the colour of the apple may be described as typically yellow-green. This stage is succeeded by a quicker fall of respiration and the apple is now full yellow colour. This fall of respiration continues, provided no fungus attack develops, on into the stage when the apple becomes brown. In fig. 2 we present these relations schematically as a time drift of the two characters. We do not predicate a very close correlation of colour and respiration since the former is determined only by surface cells while the latter is an expression of the whole mass of the apple, but the figure gives the relation which seems to be typical.

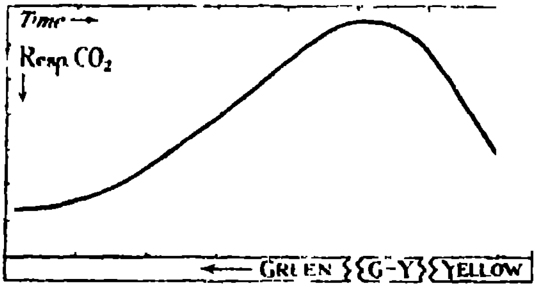

Fig. 2.—Schematic form of drift, with time, of intensity of respiration of a ripening apple passing through the senescent phase. The form of curve given here is applicable to the respiration measured initially at 22° C. when the senescence is progressing in cool storage at 2·5° C. In this generalised curve no definite values are given to either ordinate or abscissa axis, but the colour sequence which is associated with the respiration drift is indicated. Specific cases are to be dealt with in fig. 3.

Observation of a population of apples in storage teaches us at once that individual apples run through this typical drift at different rates, since some may have drifted right through to brownness when others have only reached the green-yellow stage. The implication of this is that the respiration curves of such contrasted individual apples, plotted on the same time axis, will cross one another, for the quick ripening apple will show an early maximum and an early fall, while the slow rising respiration of the slow ripening apple will cut across the fall of the other on its progress to its own later maximum. We are inclined to think that the later the maximum is attained the lower is its pitch for a given apple, as compared with the earlier maximal values of the quickly ripening apple individuals. The evidence points to all the apples having much the same low respiration values in the "mature" stage before the senescent rise sets in.

In conformity with these propositions we have fitted to our observed assembly of initial values of respiration sets of curves representing the complete respiration sequence for the whole of the senescent drift. This is carried out in fig. 3. Four of the initial points are marked by squares and these will be dealt

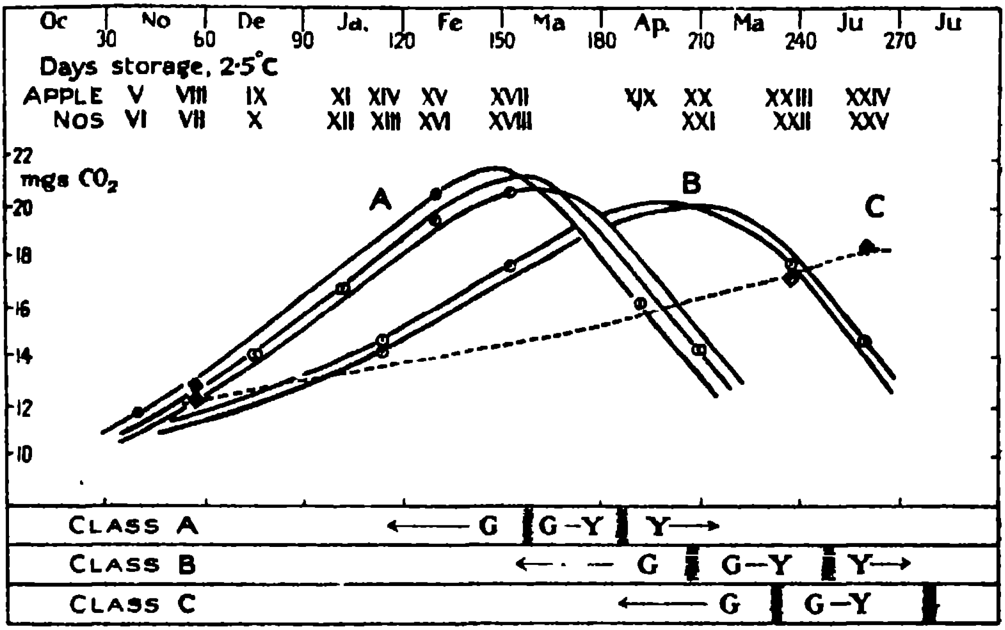

FIG. 3.—The observed initial respiration values of the 21 apples given in fig. 1 are here grouped into three separate classes A–B–C which ripen and pass through their respiration drift at three separate rates. Through each initial point has been drawn a curve of the type of fig. 2. Twelve apples are allotted to Class A and three typical curves serve to indicate their senescent drift. Five apples are allotted to the later ripening Class B and two curves serve to connect them together. The four apples of Class C are connected by a single drift line, which had not run beyond the rising phase when the investigation was ended. The identification numbers that were given to the individual apples are entered in the upper part of the figure over the individual respiration values. Where the two apples at one date have different respiration values the top number is that of the apple with the higher respiration value. The observed colour sequence noted for the apples of each class is set out below to show that the drift from green through green-yellow to yellow takes place at a different rate for each class and that the relation of these rates for the three classes is the same as that shown by the respiration rates.

with later. At present we are concerned to schematise the 17 cases represented by circles. Through these are drawn two sets of illustrative curves, and these curves imply that for any apple in the schema, the curve which passes through its observed initial value at a certain date indicates what its initial respiration would have been, had it been withdrawn from store at any other date, either earlier or later, than it was actually taken out. One striking feature of this figure is the strong suggestion of heterogeneity in the population shown by the fact that the sets of curves fall into two remote groups. Variation could easily be made in the course of the construction curves, but the absence of individuals of intermediate rates of ripening both in the rising and the falling stages could hardly be eliminated by an alternative formulation. We propose to distinguish the 12 apples that ripen quickly as representatives of Class A, while the 5 that ripen more slowly may form Class B. Below the respiration drifts are set out the colour sequence expected for Class A and for Class B according to the principles already enunciated. These sequences are based on the recorded colours as given in column 3 of the table in the Appendix.

According to this schema the scatter round the mean is very small for Class B, but only a few of this class chanced to be drawn from the population, presumably because they were in small minority. Even in Class A the scatter is not great, as the drift lines are here presented. The fact that so often two apples drawn from store at one date give nearly identical respiration values in Class A, points also to this being a real group of small scatter, rather than part of one wide common group with the remote cases of Class B.*

There are still four other apples that have not been brought to account in our treatment of Class A and Class B in fig. 3. These are VII, VIII, XXII, XXIV, represented by squares instead of circles. Apple XXIV presented us with a very high respiration very late in the chronology, and also showed a yellow-green colour. These are features of an apple near its maximal senescent respiration, which suggest that XXIV must represent a class that ripens still much later than Class B. This apple provides the first individual for our new Class C, and to this are assigned also a pair of early apples—VII and VIII. On any evidence that this chart can provide this last attribution is, of course, absolutely arbitrary, for these two apples are perfectly situated for Class A apples. But on evidence provided in Section II on air-line drifts, and confirmed in the next paper where the behaviour in nitrogen is investigated, there is no doubt whatever that VII and VIII must be segregated from their neighbours and classed with XXIV as representatives of a separate class,—C. The straight line drawn in fig. 3 from VII to XXIV would serve for the slowly rising limb of the schema of initial respiration values of this class. This line passes through apple XXII, which is also undoubtedly of Class C on similar evidence to be set out later.

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* In addition to a scatter of rates of ripening within Class A there must also be some variation between individuals with regard to the respiration value per unit of fresh weight. If this had a considerable range, the vertical divergence of lines within the nest of curves might be wholly or partly an expression of such a scatter. Introduction of this consideration might remove the intersection of curves inside each class at the peak of the schema and substitute a set of three parallel lines, but this would not affect any arguments based on the schema.

During storage, month by month, the apples are losing water, so that from this cause alone the respiration values per unit fresh weight must rise. The observed water loss is, however, not enough to make an appreciable contribution towards explanation of the observed large rise.

Such an analytic schema of three sets of lines provides some interesting situations when an apple is found at the intersection of two lines. Thus XIX might be claimed, as far as position on the chart goes, as either rising C or falling A; but the fact that it was full yellow settles it as A. Again XXII and XXIII are in the chart so balanced between rising C and falling B that we must seek other evidence. A falling B apple, not far below the maximum should be green-yellow as was XXIII. It would then have been expected that XXII, which we have referred to Class C, should have been more green than XXIII. It was not recorded at the time as more green, but only as yellow-green, though a special note was made that XXII was strikingly turgid and fresh in appearance for an apple at that late date, so that on the whole its condition supports its attribution to a rising line.

It may be mentioned that we had no schema of this type before us when the experiments were actually made, but only a growing perplexity about the association of low respiration with both the greenest and the yellowest apples. It was this perplexity that led us to select for the two apples of the late June experiment the greenest apple and the yellowest apple that could be found in the population. This gave a clue which, followed up, has led us ultimately to substitute for the perplexing configuration of fig. 1 the highly rationalised formulation of fig. 3, in which whether rightly or wrongly each point finds its place in one of three physiological classes, and also a definite position in the sequence of development of its own class. This formulation on the basis of the evidence so far produced may appear rather unsubstantial, but it will receive further support in later sections on the air-lines as well as from nitrogen effects.

It may have puzzled the reader that, whereas the whole of this section is expounded as a study of senescent drift of a population of apples stored at the temperature of 2·5° C., yet all the respiration values brought to account are for the high temperature of 22° C. The explanation of this indirect approach is that the experimental work was undertaken as a study of the effect of oxygen concentration upon apples at 22° C., and not till after it was finished was it discovered that the data supplied material for the exposition of the various analytic treatments set out in the sections of this paper. The respiration values in the present section are, however, all initial values, at 22° C., and so are determined by the physiological state of the apple at 2° C. when removed from store multiplied by the factor which gives the proper ratio for increase of the respiration rate between 2° and 22°. We have not carried out any respiration measurements at temperatures below 22° C., but the examination of such apples at various low temperatures by Drs. Kidd and West suggests that the temperature coefficient, Q_{20°, be given a value of about 8·0.

The really remarkable fact that stands out clearly, in whatever way the data are handled, is that the respiration of an isolated starving organ, at a certain stage of its drift, starts to rise considerably. We may postpone our interpretation of this phenomenon to Section III, and take up in the next section the analysis of the forms of drift of the air-lines of our population of apples.

Section II.—The Course of the Respiration Air-line of Individual Apples.

The next aspect of the respiration of apples in air at 22° C., after cold storage at 2·5° C., that we have to investigate is the general trend of the air-lines for the 21 individual apples examined, as their respiration is followed hour after hour for days. In the first section we analysed the phenomena presented by the initial respiratory values, which were found to vary from 12 to 20·6 mgs. CO_2, and we put forward a schema which introduced orderly sequences into the apparent disorder of the occurrence of the different initial values. The main conclusion was that the apples must first be sorted into representatives of some three physiological classes, A–B–C, which are characterised physiologically by ripening quickly, intermediately or slowly under the conditions of storage.

We have now to characterise and classify the different courses that the respiration of the apples run after these initial values, and see what physiological order can be introduced into this aspect also. These courses may be, for a long time, either falling, level, or rising, and we have to determine whether, for example, it is the high initial values that are associated with subsequent steep fall, while the low ones keep level, or whether the apples on the ascending limb of the A–B–C schema in fig. 3, p. 421, rise and those on the descending limb fall; or whether perhaps apples of Class C behave in a different way from Class A, and so on. For this purpose the different types of course run in air must be first brought together for an empirical comparison of forms. This is done in fig. 4. The continuous parts of the lines in the figure represent those parts of the course in which the apple was actually respiring in air, the dotted parts indicate the periods in which the apple was in other gas mixtures than air; taken together these represent the course that the respiration would have followed had the apple been kept continuously in air. The whole composite line, made up of direct observations, interpolations and some extrapolations

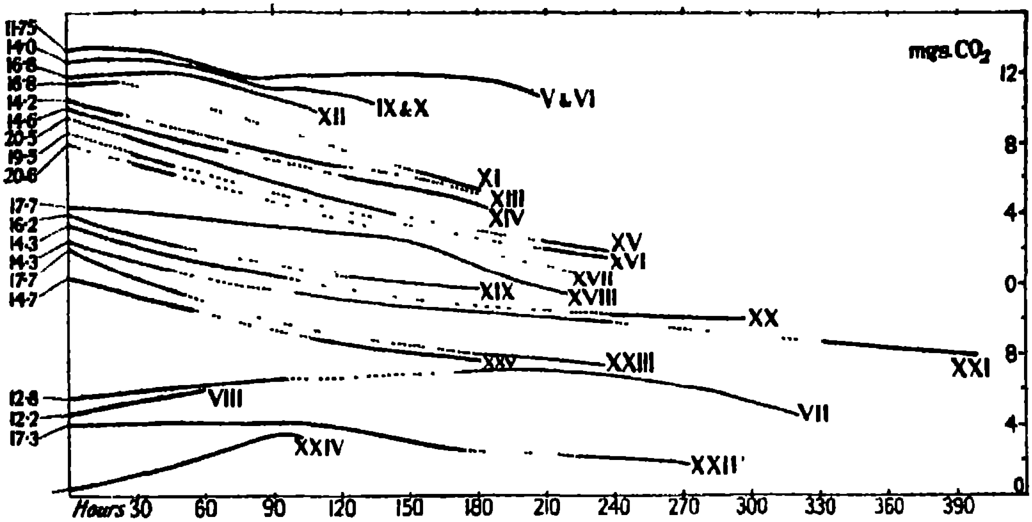

FIG. 4.—A survey of the forms of air-line drift for the 21 apples V to XXV, the abscissa axis being hours at 22° C. after removal from cool storage. The individual air-lines are not spaced out in their real relations to a single ordinate axis, but are brought close together for comparison of forms (see the table on p. 426). The ordinate scale is indicated on the right and the initial value of each air-line is outset to the left.

we speak of as the "air-line" of that apple. The derivation of these air-lines may be seen by consulting the full records of the experiments given in the Appendix to the next paper.

In fig. 4 the air-lines are arranged for comparison of forms, one over the other, as close as may be without overlapping, in a sequence that ignores ordinate values and is primarily chronological. The time axis below represents hours of respiration at 22° C. after removal from storage at 2·5° C. The respiration values which we have called the ideal initial values are noted at the beginning of each air-line. After studying these forms, from every point of view, it seemed that we could distinguish three main types, each possibly divisible into two sub-types. These are set out in tabular form and a descriptive label has been given to each type.

Classification of Air-Line Forms.

Empirical types based on form.	Sub-types.	Apples.	Fall in first 100 hours, mgs. CO_2.
I. Air lines rising initially; later declining with a steepening fall	I, *a*, fall very long delayed	VII VIII	Nil Nil
	I, *b*, fall after moderate time	XXII XXIV	Nil Nil
II. Falling air-line of composite course. Fall not more steep at first, form tending to be concave below from steepening fall.	II, *a*, course level for an initial period, then transition to fall.	V VI IX X XII XI	1·25 1·25 1·5 1·5 1·7 [3·2]
	II, *b*, approximately rectilinear downward course from beginning.	XIII XIV XV XVI XVII XVIII	3·7 3 3 4·3 3 3 3·5 1·3
III. Falling air-lines; regular continuous curves, steeper at first, slackening later.	III, *a*, initial fall moderate rate.	XIX XX XXI	3·2 3 1 3 2
	III, *b*, initial fall somewhat steeper than III, *a*.	XXIII XXV	4 0 3 6

Our next enquiry is then to see what relation these types have to the schematic relation of initial respiratory magnitudes established in Section I.

It will be seen that four of the records alone show an upward tendency and these have been grouped at the lower part of the figure. They are characterised as Type I, with the specific character that after 100 hours of respiration the value is no lower than it was initially. Ultimately these air-lines start to turn down to lower values. The rest of the air-lines have a downward tendency and are to be spoken of as falling air-lines. Among these we can distinguish two contrasted types of fall: there is one type—III—which has a very regular and characteristic form, in that it starts from the beginning with a steepish fall and proceeds perfectly smoothly, falling less and less fast, so as to present a convexity below and ending, if observed long enough, in a practically rectilinear downward slope. The standard of this is record XXI: four others closely conform to it making up the content of Type III. The remaining falling air-lines, constituting Type II, are less homogeneous and more difficult to define.

These are grouped at the top of the figure. None of them starts with a steeper fall than it shows later, and some of them hardly fall at all for the first few hours. Mostly, the course is seen to be composite in form, with a general tendency towards being concave below, but some start with a long rectilinear slope and may maintain this as long as observed. In this Type II the distinction into two sub-types is very marked.

We have undertaken a very detailed analysis of these different forms of air-line drift exhibited by individual apples of one picking, because it bears on the important question of the relative value of investigation of individual apples, as contrasted with the investigation of large representative samples of an apple population on statistical lines. It might have been that no significance could be attributed to these divergences of form shown by individual apples, and that they could only be classed as chance variations, due to causes which were too small and too numerous to be elucidated. Should this prove to be so, investigation of individual apples would be superfluous, and indeed tiresome. We consider that we have established the contrary position, and hope to show that practically all the features of these air-lines have a definite metabolic basis, and that the whole set of phenomena can be brought into one general system.

Our first business is to find out some other significant feature of apple respiration with which these types of drift can be correlated, and we will now take up their relation to the chronological sequence of initial intensities of respiration set out in the first section. Let us start with Type III as it is the most homogeneous. The apples included in this are XIX, XX, XXI, XXIII and XXV. Clearly they are late on in the chronology; but the omitted serial numbers XXII and XXIV, also very late, gave quite different records, so chronology is not everything. Reference to the schema of fig. 3 will show that all the Type III apples come on the descending slopes of the groups A and B, and also that there are no other apples on these slopes. All these apples were yellow-green or full yellow, and would be described as nearly ripe or fully ripe. We meet here a perfect correlation—as far as it goes—between type of air-line drift and position of individual apples on our A, B, C class schema. The initial respirations of this Type III vary from medium to low, but apples with these same initial magnitudes on the ascending limbs of the schema never give this type of air-line. The air-lines of XIX, XX and XXI of Class A have almost identical curves, but the air-line of XXIII falls faster at first than any of them. Also the air-line of XXV is falling slightly faster than that of XXI and diverging from it in the figure. It is therefore possible to suggest that the two on the descending limb of B make a sub-type III, *b*, just distinguishable from III, *a*, of the Class A examples

In Type I we have four records, VII, VIII, XXII and XXIV, which do not fall at all over several days, but rise, so they make a very natural class Of apple VIII we cannot say very much as it developed a fungal attack at one spot and its respiration subsequently rose rapidly, with development of visible mycelium, the initial piece alone is therefore brought into fig 4 Apple VII gives a well characterised record followed for 320 hours and rising for the first 200 hours The fall that ultimately sets in is quite unlike the fall of Type III as it starts gradually and is of increasing steepness giving a form which is concave below Two of the apples in this rising group are early apples VII and VIII, while the other two XXII and XXIV are chronologically very late, so that we get no help from this consideration The real clue is that this Type I is exactly co extensive with the Class C of fig 3 drawn up for those apples which represent a strain that ripens very slowly Within this type we are able to make a distinction based on chronology in that the late apples cease their rise at about 100 hours (sub type I, *b*) while VII keeps rising for 200 hours It will be noted that all four of these apples are on an ascending slope of initials in fig 3, and we have no knowledge of what would have happened could the research have been continued till this class of apple was fully ripe and the initials became less

We have now to consider the more complex group of Type II, which on the whole may be said to give falling curves, though not of the regular form of Type III It is clear, by exclusion, that all these apples must lie on the ascending slopes of Classes A and B of fig 3 The four earliest examples V, VI IX, X exhibit the same type of form, that of a short initial stretch, which is practically level, passing into a falling stretch getting steeper and steeper Air lines V and VI, followed for 200 hours, show a compound form in which the form of the early part is presently repeated at a lower level and there is some evidence that this is about to happen in IX and X, but the record was cut short too soon for proof Then came two contemporary apples XI and XII, of which XII conforms to the type of IX and X The form of XI is not well established since it was in 5 per cent O_2 before nitrogen from hour 24 to hour 110, as shown by the long broken line representing the interpolated part of the air line The form suggested is a long rectilinear fall which is really the form characteristic of the next sub group In the apples of sub group II, *b*, which are all later examples chronologically, we find the early level initial does not appear again, but the course may be characterised as practically a long rectilinear slope from the beginning. Leaving aside XVIII for the moment, it may be noted that the distance of fall in 100 hours is greater in this sub-class than in II, *a*. The falling tendency is, thus, more marked. The ends of these rectilinear falls for XIII, XIV, XV, XVI are rather obscure. The two former may be held to turn down more steeply but not so with the two others. Also, the general course is not strictly rectilinear but somewhat curved, though it lacks the very regular falling curve form of Class III.

The air-line of XVIII offers an arresting contrast with that of XVII which was carried on simultaneously. While XVII gives the type of form just described, XVIII starts with a long rectilinear course sloping down but very little, so that in 100 hours it is no further below its initial value than is characteristic of sub-type II, *a*. Later it turns over into a steepening slope. Referring to fig. 3, we see that the initial value of XVIII is much below that of XVII, and that it is therefore one of the apples that has been segregated as a member of Class B. The distinction of initial values between these two apples is thus fortified by the marked distinction of the forms of their air drift. The form of XVIII has several affinities with the type of II, *a*, which is chronologically earlier, as is appropriate.

We have now worked through all the forms of air-line drift in fig. 4 and see that they can be schematised into a system which finds the basis of its rationalisation not in chronology alone or in assignment to groups A, B, C alone but in a combination of these considerations. What counts is, of course, not chronology directly but its physiological aspect—grade of senescence—and as Class C is certainly ripening very much more slowly than A, and Class B may be ripening somewhat more slowly than A, then the physiologically, comparable stages of senescence are displaced relatively for the three groups. The index of senescence then becomes the position of the initial respiration on the rising and falling slopes of the class lines of fig. 3. We may then, in the succeeding paragraph, achieve a synthesis of the relation between air-line drift and degree of senescence.

The least senescent apples of our population would be the earliest ones on the up slope of the slowest ripening class, C. For this position VII is the standard and exhibits a long continued rise, rounding off to a level preliminary to what we may style a steepening fall. The next in order should be the earliest on the up slope of the quicker ripening class, A; and here we get no initial rise, but do get initial level courses, which in examples V and VI pass slowly into steepening falls, while IX and X go through this drift more quickly. The next phase that we expect after the initiation of this fall is a steepish rectilinear fall, and though there is some confusion of detail in this region we take the sub type II, b_r as representing this Apple XVIII gives support to our view, in that being Class B it should not be so senescent as the Class A apples of the same date, and it is quite clear that its form diverges in this direction, beginning with very little slope and passing over later into the steepening fall The form characteristic of the next phase of senescence is very clearly indicated by all the examples of Type III, where the initial steepish fall steadily flattens out in a very regular course giving curves which more and more approach a straight line This characterises the apples of the advanced stage of senescence, termed ripeness, and this is associated with their position on the declining slopes of Classes A and B Some support is given to the segregation of B from A in that for a given initial value of respiration an apple on B should be less senescent than one on A, and therefore start its air line with a steeper fall, which is a form a little further back on the general scheme The form of the final phase of the air-line drift is revealed only by apple XXI, which was followed for a very long time Here at the end of its record we get no further slackening of the fall, but a straight line fall of constant slope The fall of this slope is only 1 2 mg CO_2 in 100 hours, such a slope that if it were continued at this rate the respiration would reach zero only after a period of 45 days at 22° C.

As we have no very senescent apples of Class C, we cannot say whether they would conform to this schema, which fits Classes A and B

The regular succession of air-line forms during senescent drift, suffices to establish that the effects are not the chance expressions of a multitude of small indeterminable causes, but must be the outcome of simple metabolic principles In the next section we shall propose a definite schema of interpretation of these forms

Section III The Senescent Phase of Ontogeny and the Lowering of the Organisation-Resistance of the Tissues

In this laboratory various workers have studied the course of respiration in isolated plant organs of different types, and we have formulated certain fundamental principles that are to be found in action These will be the subject of a general exposition in later papers Here we limit our attention to a special phenomenon that we believe to be characteristic of the respiration of that late stage of ontogeny for which we have proposed the specific name of "the senescent phase" The special phenomenon that appears at this stage may be entitled a lowering of "the organisation-resistance" of the tissues.

We have coined the term "organisation-resistance" to express an important aspect of protoplasmic control of metabolic rate. It is quite clear that the catabolic activity of a tissue is not merely conditioned by the *amount* of reserve food-material that is present; there are times when catabolic flux is very active and times when this conversion of potential or reserve metabolites is extremely slow. This is a matter of protoplasmic organisation and it must be concluded that some of this organisation is of the nature of a resistance to reaction rate. We may picture some of this hindrance to reaction as achieved by spatial separation of the reactants by impermeable protoplasmic membranes. More significant, however, will be the adsorption or combination of one or both of the reactants by the stabilised components of the protoplasm. Phenomena of this sort are presumably associated with the control of the hydrolysis of carbohydrate reserves of the polysaccharide, disaccharide, and glucoside type.

A lowering of the normal grade of organisation-resistance would then, by definition, result in a quickening of the rate of some aspects of metabolism, more especially and significantly of those primary hydrolytic changes which bring the complex reserve and semi-reserve substances into the flux of catabolism. The particular final result of such acceleration of initial activity which interests us at present is the increase of rate of production of the effective substrate of respiration. Under prevailing conditions, in which this substrate is not already in excess, an increased production rate will reveal itself to us by an increased rate of respiration.

In place of the generalised expression—lowering of the grade of organisation-resistance—it will be preferable to use a narrower expression in this discussion, as it will be limited to respiration phenomena. Lowering of "hydrolysis-resistance" will serve our purpose or more conveniently the inverse of this which we may call increase of "hydrolysis-facility." This change takes place automatically in that late stage of the life-history of tissues which we label the senescent phase. We picture its onset as at first gradual and then progressing at an accelerating rate: later the acceleration diminishes and finally the grade of hydrolysis-facility ceases to increase and remains maximal at its new high level.

This senescent increase of facility takes place in stored apples at any temperature, but seems to have a high temperature coefficient so that it runs its course very much quicker at 22° than at 2° C., though the initial low and final high level of facility may be of identical pitch at both temperatures.

This conception of a fall in organisation-resistance and a consequent increase in catabolic changes had its origin in the search for an interpretation of the fact which we have clearly established that when the falling respiration of isolated starved organs is continuously followed, it is found that a time comes when the respiration starts spontaneously to rise again fairly rapidly in spite of continued starvation. This phenomenon of senescence will be dealt with in a more general way when we come to set out our observations on the starvation respiration of cherry laurel leaves. Here we are only concerned with its effects upon the course of the air-line drift of senescent apples.

We shall now attempt to interpret formally by a graphic schema our observed series of changing types of air-line as being the resultant expression of combined factors of senescence and starvation; the previous rate of senescence at 2° C. and that prevailing in the respiration chamber at 22° C. having both to be taken into account. In the upper part of fig. 5 are three forms of curve, Y. Z and S, representing the three significant factors. The horizontal direction of the schema represents time and the letters *b* ... *l* are points along the time drift that we shall be concerned with. The vertical direction represented by the distance between lines U and V, represents the range of change of "organisation-resistance" or "hydrolysis-facility," the level U standing for low facility and the level V for high. Before the point of time *b* the resistance is normal, the hydrolysis-facility is therefore low, so low that the substrate of respiration would be produced at a rate to give, say, 10 mgs. CO_2 per 300 grm.-hours at 22° C., or equivalently, with $Q_{10^\circ} = 8\cdot0, 1\cdot25$ mgs. CO_2 at 2° C. Let us suppose that in storage at 2° C. the senescent change sets in at time *b* and the hydrolysis-facility begins to rise slowly reaching the maximum value of the level V at time *j*. The progress of this change with time is represented by the sigmoid curve Y, and at its end at *j* the facility level of V is supposed to be just such as to give double the hydrolysis values stated for level U. At *j* the senescent change is over and the facility remains at level V on to time *l* and beyond.

Now let us suppose that the apple at time *b* should be brought from 2° C. to 22° C. as its senescent phase is beginning; it is pictured that it would senesce rapidly and rise to the level V soon after time *d*, following the sigmoid curve Z. Thereafter the level would remain at V. Suppose in contrast that the change from 2° C. to 22° C. is now carried out later, say, at time *e*. By this time the senescent change at 2° C. will be half over by progress along Y. and the rise of temperature will cause the remaining half to be carried through quickly, following the upper part of one of the Z curves, being the particular one drawn so as to intersect Y at time *e*. The full hydrolysis-facility will be reached at a time between *f* and *g*. In all, six Z curves have been drawn, one to indicate the rise at 22° C. for each of the arbitrarily selected series of cases when the apple is brought from 2° C. to 22° C. at the successive points of time *c*, *d*, *e*, *f*, *g*, *h*. In each the start of the rise of hydrolysis is along Y and the finish follows a longer or shorter track of Z.

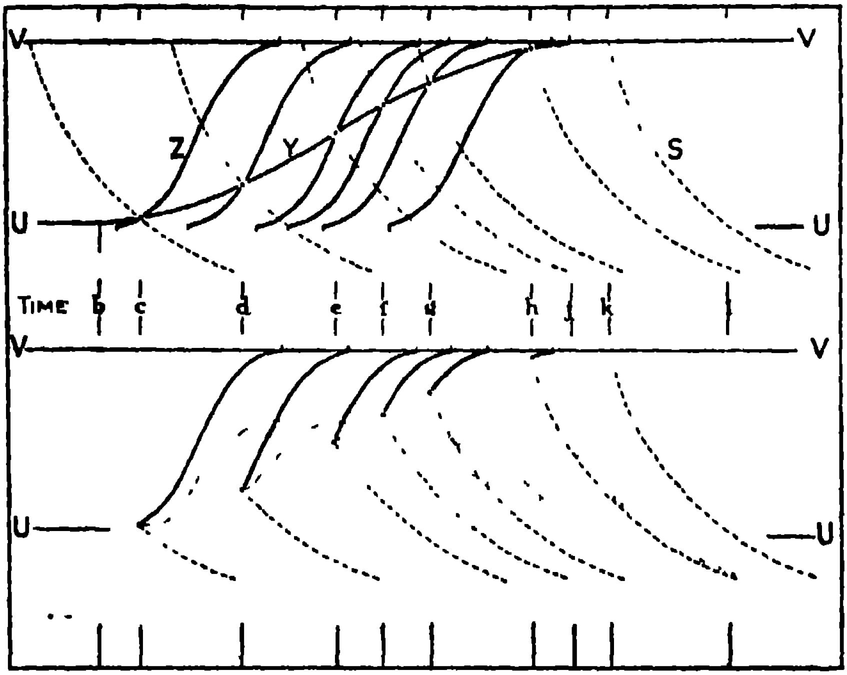

FIG. 5.—A schematic construction showing how the observed series of air-line drifts may arise as resultants of opposed tendencies. For detailed explanation see text. The letters *b* . . . *l* represent a series of points of time during the progress of senescence. The level V indicates a high grade of hydrolysis-facility and U a low grade. The upper part of the figure sets out as curves S, Z, Y, the component factors affecting the drift of respiration at any moment, while the lower part gives the resultant air-line drifts as dotted curves. Curve Y indicates the form of the slow senescent drift at 2·5° C. of hydrolytic facility from level U at time *b* to level V at time *j*, curve Z the rapid drift from U to V at 22° C. A set of six identical Z curves for 22° C. are drawn, beginning at intervals along the time axis to fit a series of cases when apples are transferred from 2° C. to 22° C. at these intervals, one after the other. The significant part of the Z curve in each case is the part which lies above the Y curve, the apple being brought from 2° C. to 22° C. at the locus of the intersection of the formal curves. The set of seven identical S curves stand for the factor of "starvation" fall of respiration at 22° C. They represent the falling tendency of respiration at 22° C. becoming effective at the point of time when the apple is brought from 2° C. to 22° C. In the lower part of the figure only the significant parts of the S and Z curves are retained, and these are set out for the six cases when the change of temperature takes place at *c*, *d*, *e*, *f*, *g*, *h*. The form of the resultant curve for each case is drawn as a dotted line. It will be noted that levels U and V are not absolute rates of CO_2-production, but stand for rates either at 2° C. or at 22° C. related to one another by the adopted value of $Q_{20°} = 3 \cdot 0$.

Were there no qualifications to be made, the implication of all this system of changing organisation-resistance would be that the respiration rate would, if kept at 2° C. continuously, rise slowly along Y, from 1·25 mgs. CO_2 at *b* to 2·50 mgs. CO_2 at *j*; or, if for contrast kept throughout at 22° C., from 10 mgs. at *b* to 20 mgs. just beyond *d*. If, however, the apple remained at 2° C. only till time *e* when its respiration would be 1·87 mgs. CO_2, and were then suddenly brought to 22° C. its respiration would at once change to $1 \cdot 87 \times 8 \cdot 0 = 15$ mgs. CO_2 and then advance rapidly to 20 mgs. CO_2 between *f* and *g*.

There is, however, a qualification of fundamental importance to be made which depends on the fact that, while the low rates of respiration that occur at 2° C. can be maintained, the high ones proper to 22° C. cannot be maintained, but tend to fall off by what may be termed "starvation." To get a pure measure of the falling starvation factor of respiration at 22° C. it is necessary to experiment outside the senescent region, at some time after *j*, such as *k*. An apple kept at 2° C. till time *k* will show respiration of 2·5 mgs. CO_2, and if then brought to 22° C. its respiration will change quickly to 20 mgs. CO_2, but cannot remain at this high level, but must fall, following the course of the broken line S, first falling fast and then slower and slower with time. The course of S represents then the pure starvation relation.

Returning to the cases where change from 2° C. to 22° C. occurs during the senescent phase at *e* or some other of the six represented points of time, then the starvation factor has to be introduced similarly at each. For this purpose identical S curves have been drawn passing through each of the points of intersection of the Y curve with the Z curves. Our components are now set out, and at any time-point where there is change from 2° C. to 22° C. then the falling curve S at that point represents the starvation factor tending to lower respiration, while the rising curve Z represents the accelerating tendency due to increasing hydrolysis-facility.

In the lower part of fig. 5 we show how these two factors interact to determine a drifting series of air-line forms which correspond in type with the series actually observed. In this lower part of the figure, those parts of the lines which we may call construction lines have been omitted, and at each of the six points of time we have left in the figure only the two opposed factors brought into being, but into opposition, by the change to 22° C. The form of the resultant curve arising from this opposition has been constructed for each of the six points by calculating the difference of the upward rise and the downward fall for a succession of short lengths of time. These forms of air-line drift are set out one by one as dotted lines.

In the early ones, while the facility is rising fast the resultant air-line drifts upwards at first, while if started at *f* it runs a level course, and if started later falls all the time. In each single case the rising facility component has a less and less effect as it nears its end at level V, and this effect becomes zero when the change is over; after this point of time the air-line follows a pure starvation course, being determined by the appropriate region of the S curve alone. The seventh curve of the series, which begins at *k* has by definition no rising component, and is the pure S curve throughout its course. The series of air-line drifts that we have synthesised in this schema presents all the observed types that we have set out in fig. 4, p. 425 The drift of *k* is the representative of Type III (see table, p. 426), but this is of course due to our definite selection of this form of starvation curve for our schema The resultant curve *d* is the analogue of Type I, *a*, in which the rise is long continued, and curve *e* the analogue of Type I, *b*. Then as we follow on, curve *f* with its level start is the analogue of Type II, *a*, while curve *g* giving a rectilinear fall represents Type II, *b*.

Our work on the apples was not begun until they had been in storage six weeks, and we consider that the absence of any observed air-lines rising so steeply as the resultant curve *c* is due to the fact that this type occurs only in the earliest stages present in October.

It should be stated that this schema is not an attempt to reconstruct the actual air-lines observed but only the *types* in their proper serial succession. The curves Z, Y, S, employed in building up the schema were not arrived at by careful trial of form to give the best fit, but were drawn freehand without subsequent adjustment. Nevertheless it is obvious that, for a given time axis in the schema, the mutual relation of the slopes of the adopted Z and S curves determines the synthetic form of the air-line drifts. Had S been nearly as steep as a vertical line, or nearly as flat as a horizontal line, Z being unchanged, then the air-line forms would come out very different. Some narrow range of relation between steepness of S and Z has therefore really been predicated in the application of the schema. More general aspects of the significance and forms of starvation curves have to be taken up in a later paper.

Further, it will have been noticed that nothing has been said, so far, about the existence of a starvation curve component at 2° C. as well as at 22° C. We have tacitly assumed that this component at 2° C. is so flat in form, that it involves so little decline of respiration rate with time, that it has a negligible effect upon the slope of the air-line. At this temperature, then, the rising facility curve is held to express itself fully stage by stage in the observed rate of respiration. We have made no respiration measurements at such low temperatures, but this aspect of apple metabolism has been studied by Kidd and West, and the relations we have indicated may be put on a more exact basis when their work and ours come to be correlated.

In all this matter we have treated the individual apple as a whole and have not stated whether the drifts observed are to be considered as true for each individual cell of the apple or only as a statistical truth for the drift of the whole population of cells making up the tissue.

At least it can be said that our schema fits the facts, and that it provides a new conception which helps us to interpret the complex behaviour of respiration phenomena, by assuming that the organisation-resistance of tissues is not constant but is capable of undergoing spontaneous change. In other work we shall show that the resistance can be altered by experimental treatment.

Section IV.—The Initial Effect of Change of Temperature.

When an apple is heated up from 2° C. to 22° C. the main effect upon the respiration is an increase of rate to somewhere about eight-fold. The course of this rising respiration, hour by hour, does not, however, proceed to the new rate by a continuously rising curve of the form that a simple transition from a low steady rate to a higher steady rate would give, but the transition record may exhibit a definite peak, so that the CO_2-production is, for a time, in excess of the air-line rate that it will attain later on. In the present section we have to examine the early parts of our records rather carefully for evidence of the presence and magnitude of this effect.

For this purpose these parts of the records have all been brought together in the two columns of fig. 6, alined at zero hour; and certain construction lines have been drawn upon them to facilitate their examination. For clearness, in this figure, each record is presented by its pair of "contour lines" (see p. 416) and the actual readings lying between them are omitted. In the earliest steeply rising hours the contour lines lie close together but later they are at the standard distance apart due to the fluctuation of the respiration (see p. 416). In this later region a median line is drawn between the contour lines giving the direction of drift of the specific air-line of that individual apple. We have extrapolated each of these air-lines back to zero hour to provide one of our two construction lines. At the top of the figure, two in each column, will be seen the four apple records which exhibit a definitely rising air-line, and in three of these VII, VIII and XXIV the existence of temporary excess values of respiration is very clear. After the initial maximum the

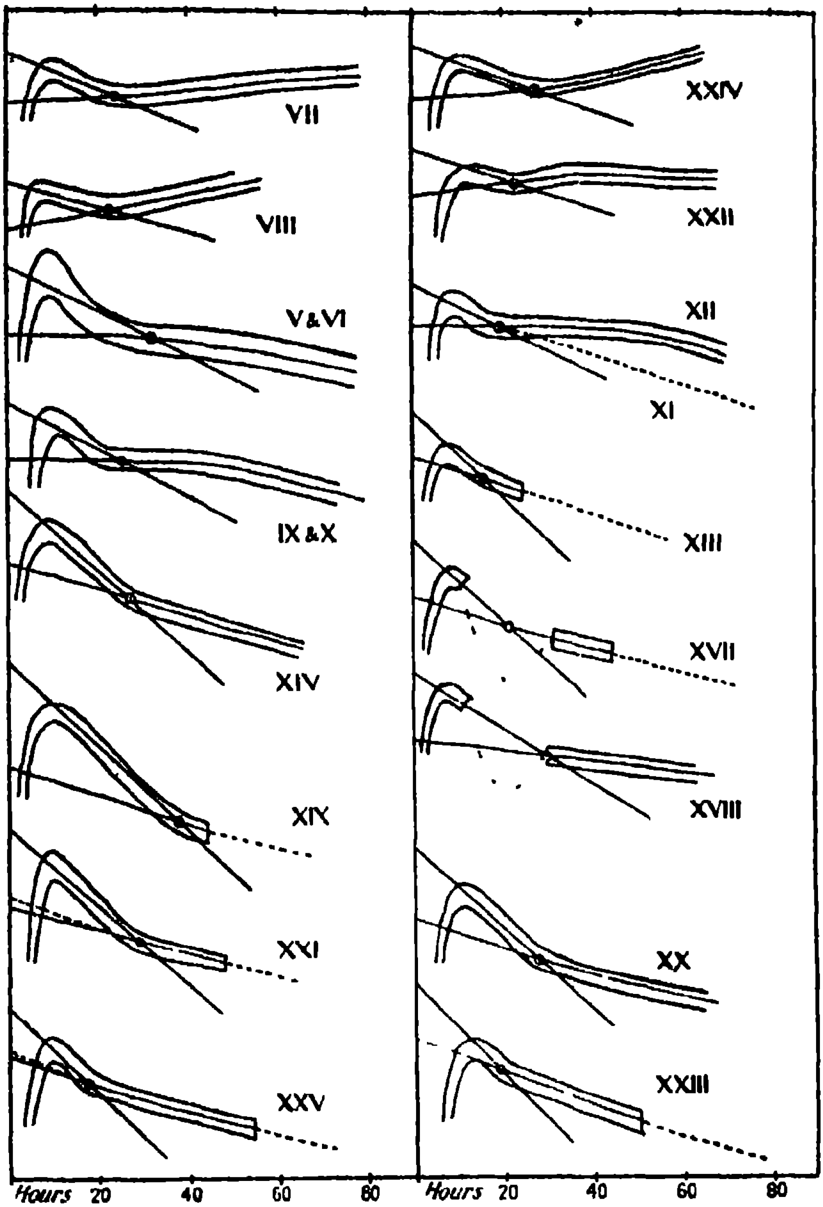

FIG. 6.—The initial hours of all the records brought together for examination of the "initial temperature effect" on change from 2·5° C. to 22° C. The low readings of the first six hours are omitted to save space. The actual course of respiration is defined by the track of the double contour lines. The ordinate scale and the values of the single readings can be seen in the charts of the records in the Appendix to the next paper. The circle on each record marks the "inflexion point" and indicates the end of the initial effect. The median air-line is extrapolated to zero hour. Where this air-line has a marked curvature the extrapolated curve is given as a broken line, and the continuous straight line near it is a construction line drawn as a tangent to the curve at the inflexion point. A second construction line is the straight line drawn through the inflexion point, along the median track of the record before the inflexion point. For the application of these construction lines see the text. Records XVII and XVIII are interrupted by a failure of temperature control from hours 12 to 32 (see the Appendix).

record shows a series of declining values, which present the curious feature of lying on a straight line, and this presently brings the record on to the true air-line. Here then there is a well-marked "inflection point" on the record as the respiration values thence proceed to rise along the air-line. As a second construction line in the figure, the median line of the observed falling slope has been continued back to zero hour, and onwards in the other direction to define its slope more clearly. We thus get superposed on the record a pair of construction lines intersecting at the inflexion point. The angles at intersection appear to be constant in all the well-marked cases, being 25° and 155° with the relation of axes adopted in this graphic presentation. The slope of the passing-off of the initial temperature effect is not then a constant slope to the horizontal, but a constant difference of slope above the slope of the air-line of the individual apple. One implication of this relation is, of course, that the higher the initial maximum lies above the extrapolated air-line, the later it will be before the inflexion point is reached. The time values for these relations are set out in the Table of the Appendix to the next paper.

It now becomes interesting to consider our numerous records in which the air-lines show a well-marked fall as a general character, *see* Section II. These eight records are grouped in the lower part of fig. 6, four in each column. Here we can hardly expect to find so obvious a contrast [illegible] direction at the inflexion point since both lines will be falling ones. The method we have adopted for interrogating each record is that of superposing on it tracing paper on which our two lines, intersecting at the standard angle, have been drawn. One line is put to coincide with the course of the air-line and shifted along it to see if the intersecting line comes to lie on the median course of the falling slope of the change of temperature effect. It will be seen that apples XIV, XX and XXI give very convincing evidence of the existence of a big effect when ruled up in this way, and the intersection point is correspondingly late, 27 to 29 hours; see the time values in the table in the Appendix.

Two other records XXIII and XXV show small effects, about which one cannot be very certain, the inflexion point coming at 18 to 19 hours. Apple XIX appears to have an exceptionally large effect with inflexion point at 38 hours, but this apple was transferred to nitrogen at hour 44 so the air-line course after the inflexion point is not very securely located; on general evidence we believe the dotted track in the figure is the course it would have followed had it been kept in air. Apples XVII and XVIII, investigated together, both show an unfortunate break in their records, due to an accidental fall of temperature in the bath soon after the point of time when the maximum of the effect had developed at hour 9. The effect of this lowering of the respiration was not fully recovered from till hour 32, and a single median track only is drawn for the respiration of this distorted period. The direction of the subsequent air-lines for these two experiments enables us to extrapolate to zero with confidence and our cross-lines can be fitted to the two fragments in a way that is satisfactory enough. We may conclude that with all these cases of respiration the same type of change of temperature effect occurs as with rising air-lines.

We have still left a group of four records in which the air line starts with a short level course. These, which appear in the middle of fig. 6, show a well-marked temperature effect, and the same construction of the intersecting lines has been superposed upon the records. Though there is no doubt of the presence of the effect in records V and VI, IX and X yet the fit of the straight lines is not so convincing as in the other cases. Both these two records are composite records, that is to say, that all the readings for two separate experiments V and VI or IX and X, carried out simultaneously, have been plotted as one record. In each case the readings of the pair intermingle and their collective fluctuations fall within contour lines which are not twice as far apart as the normal spacing for a single apple ; see the records in the charts of the Appendix. It will be noted that the falling slope from the maximum to the inflexion point in these four apples can hardly be described as a straight line course, but tends to be a declining curve, which indeed is the form to be expected *a priori* in all cases were the fall to the air-line to be of an exponential nature. Apples XI and XII also start with a short level air-line but the temperature effect is not very large and so is not very well defined, but here also there is some indication of a slope from the maximum which is convex below rather than rectilinear. We would conclude that in the group of apples which start with a level air-line the same type of temperature effect takes place as in the other two groups, but that here we have a variation of form of the slope from the maximum. It interests us, in our endeavour to analyse out all the details of behaviour of apple respiration that this group of apples is not a random one but is the group of apples with air-lines of Type II, *a*, on p. 426.

The only records that we have not yet referred to are XIII, which is so short that it is ill-defined but appears to exhibit a minimal special effect, and the pair XV and XVI for which, through an accident, we have no early readings at all. The change of temperature effect, then, may be held to be always present in our apples, but its magnitude varies a good deal. Its uniformity of form is striking, seeing that it keeps true to type, whether respiration, fundamentally, is either rising or falling, while the departure from the majority type is but slight with level respiration. Possible interpretations of this effect will be examined in the next section.

Section V.—Change of Temperature and CO_2-production.

Let us first consider the various factors within an apple that might express themselves by a transient excess evolution of CO_2 to the external air-current when the temperature is quickly raised from 2° C. to 22° C. The effects of this heating up upon CO_2-evolution may be divided into physical and metabolic; and two processes may be considered under each heading.

Physical Processes: Alteration of Equilibrium of Solution, Adsorption and Loose Chemical Union of CO_2.—At the initial temperature of 2° C. the apples start with a certain percentage of CO_2 in their air spaces plus so much in solution in water in equilibrium with this, so much CO_2 adsorbed and so much loosely united as bicarbonates, etc. All these states are reversible ones, and the equilibrium point of each will be shifted to a lower value in the sorbed phase by a rise of 20° C. in the temperature. From each state then CO_2 will tend to be liberated by a rise of temperature, and it might be thought that the excess production of CO_2 that we have noted was simply an outcome of the shift of these equilibria. Thus the absorption coefficient of water, when heated from 2° C. to 22° C., falls from 1·6 to 0·8; so that the water would give off half its dissolved CO_2 provided it continued in contact with the same external partial pressure of CO_2. On further consideration of the conditions established in the heated-up apple, it appears, however, that this provision is not complied with. For an apple at 22° C. gives off steadily by respiration to the air current some 8·0 times as much CO_2 as the apple at 2° C., and as the ultimate stage in this escape is inevitably a diffusion gradient across the surface of the apple there must be maintained a much higher concentration of CO_2 inside this surface at 22° than at 2° C. for such a transference by diffusion. The increase of diffusivity and decrease of viscosity in the medium are not very great for this rise of 20° C.

In accordance with this expectation it has been found by work to be published subsequently that the internal atmosphere of an apple contains at least five times as much CO_2 at the higher temperature. The rise of internal partial pressure of CO_2 is thus so great that it actually overbalances the shifting of the solution equilibrium point. It follows that the water in a respiring apple at 22° C. should contain in solution at least $\frac{0{\cdot}8}{1{\cdot}6} \times 5 = 2{\cdot}5$ times as much

CO_2 as at 2° C. We cannot, then, attribute any of the evolved excess to this source.

Another possible physical source of CO_2 would be the liberation of adsorbed CO_2 by the rise of temperature. The same general considerations must be borne in mind as for the case of solution in water. The actuality of liberation will depend upon whether the known rise of partial pressure of CO_2 within the apple to about five-fold is adequate to balance out the lowered affinity of the adsorbent. There is no quantitative knowledge available for the apple; also its content of organic matter is low. It is to processes of a metabolic nature that we incline, therefore, to attribute the observed change of temperature effect. To the two metabolic processes that suggest themselves we may give the names of "carbohydrate equilibrium effect" and "intermediate compound effect."

The Carbohydrate Equilibrium Effect.—It is well established that such a change of temperature as our apples are subjected to, produces a shift in what is generally called the starch-sugar equilibrium relation, so that sugars accumulate at the expense of starch during the time the tissues are kept at low temperatures. When the tissues are brought to a higher temperature this accumulation of sugar is reconverted to starch so that initially the sugar concentration is in excess for the high temperature state but rapidly declines. We should expect, as a result of this sugar behaviour, high initial respiration at 22° C., falling with time as the sugar falls. This respiration effect is very marked with potatoes on change of temperature and the apple effect might be of this nature. There is also evidence that such temperature changes affect the relations between cane sugar and hexoses. The respiratory effect in apples might be due to this metabolic cause.

The Intermediate Compound Effect.—Picturing respiration as fundamentally a sequence of linked reactions its progress at a steady state must be associated with definite equilibrated concentrations of the series of intermediate compounds that constitute the reactants. With rise of temperature and the change from a steady state at 2° C. to a new steady state at 22° C. there are possibilities of transient excesses of CO_2-production according to the varying effect of the rise of temperature upon the component reaction velocities.

We have before us, then, three possible mechanisms that may contribute to the excess production; the adsorptive, which is outside the cell's metabolism; the carbohydrate equilibrium which is metabolic but outside the essential respiratory nexus; and the intermediate respiratory compound, which is of the essence of the respiration itself. It would seem that these three mechanisms have sufficient *differentia* for us to hope, later, to distinguish between them. All we can do at present, as a contribution towards solution of this problem, is to survey the set of effects that we have recorded and determine whether the variations of magnitude and timing that they present give any helpful indications. Some indication might be obtained should a clear correlation appear between the varying size of these effects and some other feature of the apple respiration. Were the effects of identical magnitude in all cases, then this relation might be held to support the physical interpretation. Should there be a close correlation between intensity of individual respiration and the magnitude of the effect, then the intermediate respiratory compound view would find support. If on the contrary the effects were large at early stages of senescence and steadily declined chronologically so that there was a correlation with what we may call "starvation," then this would support the carbohydrate equilibrium interpretation. We shall see presently that no single one of these possible correlations dominates the whole situation.

One considerable difficulty that stands between the observer and elucidation of these transitional phenomena is that the intensity and duration of an intracellular production of CO_2 is so much distorted when the observed signs of it are only the intensity and duration of outward escape of CO_2 by diffusion through the surface of the massive tissues of an apple. Should a few cubic centimetres of CO_2 be suddenly produced in excess by the respiratory mechanism, which before and after maintained a steady state, then this would not manifest itself outside as a sudden output of CO_2 of identical timing and intensity, but as an output which might rise fairly quickly to a maximum but would decline quite slowly again towards the steady rate, owing to diffusive lag. The decline would take the form of a "logarithmic curve." It is clear then that when we observe the CO_2 production of an apple falling in a curve of this sort from a high level to a lower level, and taking many hours to complete the falling transition, we cannot conclude that any actual internal production of CO_2 has been continuing at a heightened rate beyond the point of time at which the high level ceased and the fall began. The observed escape of CO_2 may be described as a distorted anamorph of the production.

A measure of the total magnitude of the excess internal production of CO_2 is, of course, given by the total excess escape of CO_2, provided our graphic records provide a sufficiently certain base-line above which to measure the area expressing the excess escape. This certainty we do not possess in dealing with the initial temperature effect in the apples, but we may take a summary survey of the magnitudes in excess of the base-line which is provided by the theoretical extrapolated course of the air line This survey is represented in fig 7 where the common base line stand for the air line level and each magnitude is represented as the area of a triangle emerging above this base The vertical side of each triangle gives the height above the air line value which the CO_2 output reaches at its maximum and the length of the triangle along the base line measures the time between the maximum of the effect and its extinction at the inflexion point on the air line

In this figure are assembled a series of these triangles and we have to enquire whether their respective sizes fall into any simple system After a careful survey of the cases it seems to us that the distribution of magnitudes has a dual determination and that it is first of all essential to take account once more of the A B C Class schema evolved in the first section In fig 7 the individual cases have been grouped into three rows for the three classes and spaced along the rows according to their positions on the rising and falling slopes of their respective classes In Class A we find apples V and VI IX and X and XI and XII which are on the rising slope presenting a clear chronological drift of declining magnitude of the special effect so that here there is an inverse relation to rising magnitude of general respiration and a direct

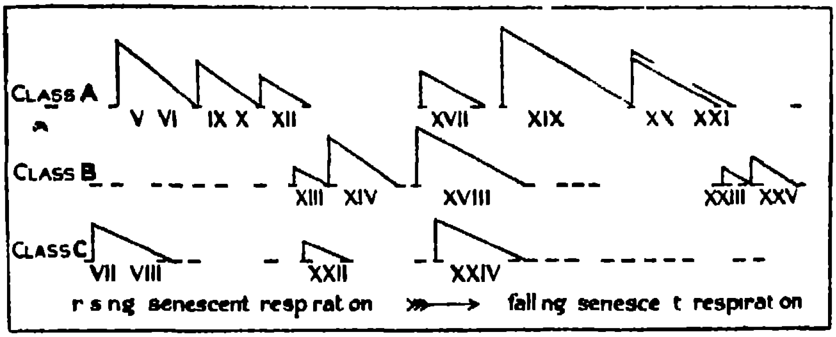

Fig 7 —A diagrammatic presentation of the relative magnitudes of the excess CO_2 production in the change of temperature effect for the 19 recorded cases The areas of the triangles provide the relative measures of magnitude the vertical axis being intensity and the horizontal duration The base of each triangle measures the time of the falling part of the effect from the observed maximum to the end at the inflexion point The vertical of each triangle measures the intensity of the excess production above the arbitrarily adopted values of the ideal air line the true base line from which the excess should be measured cannot at present be determined The triangles are arranged in three series corresponding to the three classes of rate of ripening and are spaced out within the classes so as to bring corresponding states of senescence over one another Thus arrayed we find indication of an initial declining series followed by a reversion to high magnitudes and then a second declining series

relation to progressive starvation. Further along on the falling slope of Class A we have apples XIX, XX, XXI, and these show another declining series with time and progressive starvation, but here, in contrast, the general respiration pitch is falling with time. Between these two groups the special effect must have increased in magnitude, but the only available apple between is XVII, so there is not clear evidence of the real course of the facts in this region. In Class B we have a suggestion of a parallel to the second falling group in the relation of the series XVIII to XXIII and XXV. Apple XIII provides a very low value in the middle region. Class C with its long continued rising slope provides a diminishing series of effects from VII and VIII to XXII followed by the large effect of XXIV.

Viewed in this way it may be held that the whole set of effects shows evidence of decreasing magnitude with progressive starvation up to the maximum of initial respiration, but that here the end of the senescent rise of general respiration brings about metabolic change to a new basis, and the magnitudes rise only to decline once more as starvation progresses further. For this interpretation of the magnitudes of the initial temperature effects as a double series of drifts we do not claim even high probability, but it provides suggestions for future examination. At least it seems clear that the observations cannot be rationalised without recognition of class grouping and of the main phases of senescent drift.

There are a good many factors that might be significant in the interpretation of these effects. We have had the advantage of additional suggestions from our colleague Mr. Briggs in discussing this and other biophysical aspects of the present stage of our general analysis of respiratory phenomena.

Conclusion.

In this paper there has been taken up the task of treating analytically the respiratory phenomena presented by a collection of apples in storage which are slowly ripening during a period of eight months. The general metabolic phenomenon that is proceeding in such a population is a steady drift through a definite ontogenetic phase, which, to distinguish it from the phases of adolescence and maturity, we term the senescent phase. This phase consists, in essence, of a fundamental change in the organisation of the tissues, which we describe as a lowering of the normal organisation-resistance, so that hydrolysis of reserve and semi-reserve substances proceeds at a faster rate than in the mature phase. This change leads to a greater production of the effective substrate for respiration, and so to an increased production of CO_2. When this senescent change has completed itself respiration falls in the direction of zero by the natural starvation condition that is present in an isolated plant organ.

Our procedure for the analytic study of this stored population kept at about 2·5° C. has been to remove from it individual apples at intervals and to examine their respiration at 22° C. The primary quantitative features that the respiration of each individual presents are two: (1) the initial value which is the measure of its physiological state when removed from store, and (2) the form of the subsequent course of respiration hour after hour when continued at 22° C. These two features exhibited a great variety of magnitudes and forms, and at first sight the distribution of these in chronological series, apple after apple, seemed to be almost random. After careful critical collation of all the forms, evidence of systematic drift emerged.

Finally the conclusion was forced upon us that there were certainly two, and possibly three, physiological classes of apples present, which classes ripened at different rates so that chronology and metabolic drift of the whole population did not move together. On correcting for this we could formulate the characteristic phases of the metabolic senescent drift, and show their succession in each class. We found then that the observed respiration of an apple is an integration of two independent and opposed processes that are at work in senescence. One is the starvation drift at 22° C., tending continually to lower the respiration, while the other, tending to accelerate the respiration is the lowering of organisation-resistance, expressed in this connection as rise of hydrolysis-facility.

In the third section of the paper a scheme has been presented in which these tendencies are brought to account, and it is shown that the observed types of phenomena can be reconstructed in this way. We would not claim that the interpretations that we have put forward in this paper are fully established by the evidence produced. We set ourselves the task of examining every detail of respiratory behaviour, and after collation of the details drawing up a schematic interpretation. As the apples were steadily drifting into new metabolic states, one after another, all the time, it was not possible to go back and recapture any precise set of conditions. Experimental verification of a hypothesis was therefore not a practicable procedure. Only by working over the whole field again in another year will it be determined how far the details of interpretation can be maintained.

Analytic Studies in Plant Respiration. II.—The Respiration of Apples in Nitrogen and its Relation to Respiration in Air.

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Introduction.

The previous paper of this series contains a study of the respiration of a set of 21 apples brought from cold storage at 2·5° C. and examined in the laboratory at 22° C. The complex phenomena presented by the respiration while in a current of ordinary air were alone dealt with in that paper, but these same individual apples were also investigated in nitrogen and in various percentages of oxygen. In the present paper the respiration data obtained in nitrogen are to be examined, while a subsequent paper will take up the phenomena presented in concentrations of oxygen.

The complexities of CO_2-production in nitrogen are no less than those already studied in air, though they bring us into a new field in which the oxidation factor is excluded. The behaviour of a number of individual apples alternately in air and nitrogen is recorded by long series of continuous observations, and these call for detailed analysis.

So many relations which seem both novel and significant emerge from this analysis, that we propose to set out our study of them formally in three successive parts. Part I deals with the actual course of CO_2-production in nitrogen and presents our observations empirically as a set of "nitrogen effects." In Part II, our data are presented in the way usually adopted by workers in this field, in that the magnitudes of CO_2-production in nitrogen are set out as ratios to the CO_2-production in air. This stage of analysis points the way to a more fundamental examination, which is carried out in Part III. In that part an attempt will be made to interpret the observed phenomena as manifestations of the working of a catalytic system of respiratory reactions, bringing into account both the main aspects of respiration, namely, those of oxidation and those of carbohydrate consumption This part is presented as the third paper of this series.

Experimental Procedure.—The general conditions of experimentation are fully described in the first paper, and need not be repeated. For the nitrogen experiments the procedure adopted was to disconnect the air stream through the apple chamber and to substitute a stream from a cylinder of compressed nitrogen, the flow being adjusted by a valve to give the standard rate of 1500 c.c. per hour. The stream of gas issuing from the chambers passed to a set of Pettenkofer tubes in which the CO_2 was absorbed by baryta; the production of CO_2 is expressed as milligrammes per 3 hours per 100 gms. fresh weight of apple.

It was found that some cylinders of commercial nitrogen contain as much as 1 per cent. oxygen. As very small partial pressures of oxygen give quite different results from oxygen free nitrogen, the gas had to be freed from traces of oxygen by slow passage over heated copper gauze on its way to the chamber. This gauze was contained in a silica tube in an electric furnace heated continuously to 700° C. After the furnace came a Pettenkofer tube of baryta solution to remove any traces of CO_2 before the gas passed to the apple chamber.

PART I.—AN EMPIRICAL SURVEY OF THE EFFECTS OF NITROGEN UPON CO_2-PRODUCTION IN APPLES.

The Records.—Among the full records of the respiration of individual apples set out in the Appendix to this paper will be found 10 cases in which individual apples have been subjected to nitrogen for periods varying from 40 to 100 hours and their CO_2-production compared with that in air. Nine of these cases bear, in chronological order, the numbers VII, XI, XIX, XXI, *a*, XXI, *b*, XXII, XXIII, XXIV and XXV.* In this list appear no more than 2 out of the first 18 apples, while 5 out of the last 6 were examined in nitrogen. Behind this unequal distribution lies the story of a quite unexpected complication. The record of the first apple treated with nitrogen, No. VII, was evidently of quite the same type as we were familiar with from a detailed study of the behaviour of Cherry Laurel leaves in nitrogen, so this section of the work was dropped for experiments on small percentages of oxygen. Not until apple XIX was a second experiment made on respiration in nitrogen, and to our surprise the type of record obtained was quite different from that of VII. This divergence concentrated our attention on nitrogen for the rest of the series, but the lack of more nitrogen records in the first part of the series leaves several problems unsolved for the present.

The numerous nitrogen experiments made after XIX showed that there were really two well-characterised types of nitrogen effect.

Of the nine records in nitrogen to be considered, seven were cases in which the nitrogen treatment was both preceded and followed by a period in air, and these must first occupy our attention, leaving the two complicated cases in which the treatment was different for Section III.

Fig. 1 presents a chart of the directly significant parts of the records of these seven apples, arranged so that the hours of beginning of nitrogen for all cases are in alinement. The chronological order of the experiments was that of the Roman numerals attached to them, but they are not arranged in that sequence but are grouped into the two different types of nitrogen effect. It will be seen that the top three in the chart differ in general appearance from the bottom four. One of the main puzzles, on the first survey of results, was to find out some explanation of why, apparently at random, one apple gave one type and another the other type. The difference could not be directly correlated with immaturity and maturity, because the first apple examined in nitrogen, VII, in November, gave the same type as two of the last examined (XXII and XXIV) in June of the next year. Those examined intermediately (XIX, XXI, *a*, and XXI, *b*) gave the other type of reaction. Nor could the difference be correlated with differences of high and low magnitudes of respiration in air, for VII has a low air value, while XXII and XXIV have high ones. Apple XXV alone gave a reaction that could, to some slight degree, be regarded as intermediate between the two types.

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* The tenth case, that of apple VIII was exceptional, being attacked by a fungus mycelium, and the complex problem of the effect of nitrogen on the respiration of the combined system is postponed to Section IV.

The solution came, not from a study of the nitrogen records themselves but from a detailed analysis of the behaviour of the respiration of individual

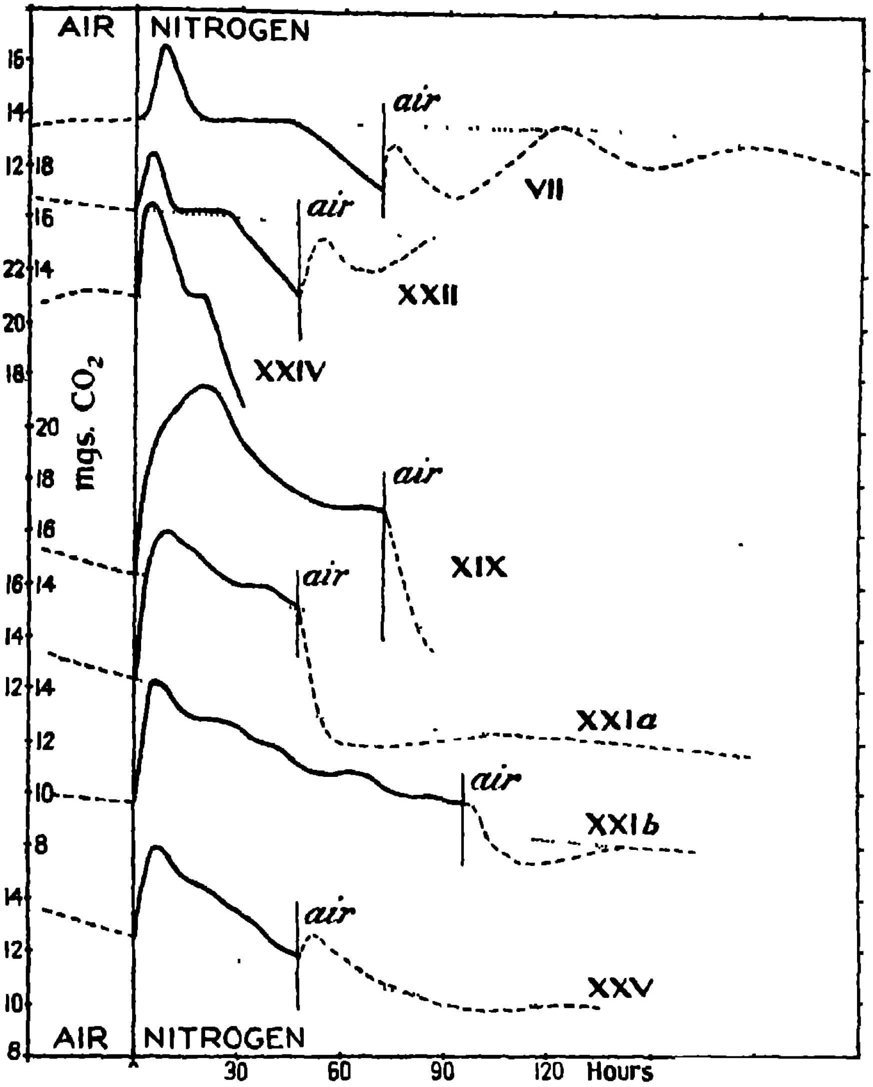

Fig. 1.—Presentation of seven examples of the effect of nitrogen upon CO_2-production, being all the observed cases in which nitrogen was preceded by air. All cases alined for hour of entry into nitrogen. The heavy continuous line is the record of the CO_2 in nitrogen. The broken line before and after nitrogen is the record of CO_2 in air. The dotted line gives the "air-line respiration" by interpolation. For the full records, see the charts in the Appendix. Groups of the appropriate ordinate values are given to the left. The top three records are those of Class C apples; the bottom four those of Classes A and B.

apples in air. This has been carried out in the first paper of this series, where the conclusion has been arrived at that these apples were not really a homogeneous population, but that they must be segregated into representatives of certainly two, and probably three, physiologically distinct classes. Class A, distinguished by quick ripening, is sharply marked off from Class C, which ripens very slowly, while there is some evidence for a third Class, B, which ripens at an intermediate rate. It is a great support to this conclusion to find that this classification gives the key to the distribution of the two types of nitrogen effect.

We have now to bring into prominence the striking differences between the two types by characterising each of them. In the records brought together in fig. 1, the abscissal hours are numbered from the change into nitrogen from air; and the continuous line gives the course of the CO_2-production that results, expressed in milligrammes CO_2 per 300 grm.-hours of fresh weight of apple. The figures on the ordinate axis adjacent to each record give a group of the numerical values appropriate to that particular record. The broken line for 30 hours before zero time gives the course of respiration in air during that period; while the broken line after the continuous line marks the course of respiration in air when the apple is returned to this gas. The fine dotted line which continues the first broken line through the period in nitrogen is the course that, we conclude, the respiration would have followed in air had not nitrogen been substituted for it. These pieces of the line together constitute the "air-line" of the record. It will be noticed that the interpolation only joins the actual air values some way on after the apple is back in air again —not till there is evidence that the respiration is really in adjustment with air again and the transitional after-effects have passed away.

We may now consider the two types of nitrogen effect in separate sections.

Section I.—The Nitrogen Effect with Apples of Class C.

To this class belong the records of apples VII, XXII and XXIV, presented at the top of fig. 1. In all three of these records it will be seen that when the apple is subjected to a current of pure nitrogen the resulting change of CO_2-production follows a highly characteristic course. It rises at once for a few hours and then falls sharply to a level identical with that of the air-line; after this level value has been maintained for a longer or shorter period of time, there sets in, quite suddenly, a rapid decline of the respiration in a falling straight-line course.

The whole observed record for apple VII lasted no less than 11 days of continuous estimations, each of 3 hours' duration (see the Appendix for the full record). Before nitrogen was given the apple had been observed in air already for 94 hours, but only 30 hours are brought to this special figure. The air record before nitrogen is nearly level, with a faint rise in it. After the change to nitrogen there is a rapid rise, giving a crest with a maximum of 16·6 about 9 hours after the change. This outburst of CO_2 subsides as quickly as it rose, and in 20 hours from the beginning of nitrogen it is down to the original level that it held in air. At this level it now remains from hour 20 to hour 48, when it starts a new change and declines fast in a straight line, reaching 11·3 mgs. at hour 68. At this point its nitrogen experience was terminated by a current of air.

The air record after nitrogen never rises above the "air-line" and there is apparently a good deal of metabolic disturbance following the exhibition of nitrogen in the record of VII, for the CO_2 values follow a fluctuating course, drifting first up, then slowly down and up again, before the air-line is reached once more, in a couple of days. It is not proposed to interrupt the exposition of these nitrogen effects by justifying in detail the course given in the figure to each air-line. These are considered in the notes to the Appendix containing the whole records.

Not till apple XXII did we get another record belonging to the type of Class C. The respiration in air was 16·1 when nitrogen was given, and rose to 18·2 in 5 hours, it then fell to 16·1, completing the hump in 12 hours. After this there comes a level course, lying on the air-line, lasting till hour 28, at which time the steep downward track sets in, reaching 12·7 at hour 50, when air was given again. The behaviour after air is identical with that of VII as far as it was followed. Like VII, the fluctuations start with a sharp rise, but not enough to bring the record above the air-line. The chief difference from VII is that the level second phase in nitrogen is so much briefer in XXII, 16 hours instead of 27. This apple XXII was green-yellow and nearing maturity, while VII, taken six months before, was unripe green. This suggests a decreasing resistance to anaerobic conditions with advancing senescent development.

Seeing how comparatively rare this type of nitrogen effect had been up to experiment XXII, it was surprising to find that one of the next pair of apples to which nitrogen was given—XXIV--belonged again to this type. The respiration before nitrogen had been followed for 100 hours and had been rising for 85 hours; then it became nearly level for 15 hours, reaching 20·8 when nitrogen was given. We now get the characteristic sharp hump of 16 hours' duration, but here rising higher, up to 24·4. This was followed by signs of a level phase on the presumed air-line, but this only lasted

6 hours and suddenly down plunged the line of CO_2-production. In this case it was decided to determine whether this fall was in any way a toxic effect, for no apple had been killed by nitrogen among the previous experiments. Nitrogen was therefore kept on for 112 hours (see the full record) and the fall continued straight down to the value of 5·3 and then sloped off less steeply to 2·4. When air was readmitted the respiration only rose to 4·0, so that this apple has been practically killed by absence of oxygen for 112 hours.

With these three cases it is difficult to avoid the conclusion that as apples of this type slowly ripen, their metabolic working changes so that they become in some way decreasingly resistant to nitrogen treatment.

One more nitrogen experiment—XXV—was tried before the work had to be brought to an end, but this turned out not to belong to the type of Class C.

The three apples VII, XXII and XXIV are, then, the cases on which a special type of nitrogen effect was established. It was only later that it was made out, from study of the air records and apple colour, that there were classes of apples of different ripening rates, and then it was seen that the slow-ripening Class C was just co-extensive with the group that gave this Cherry Laurel-leaf type of nitrogen reaction, and could not resist, without depression below the air-line, long withdrawal of oxygen.

It may be mentioned here that on consideration of the early apple VIII, which became attacked with mycelium and is therefore to be dealt with separately in Section IV, we came to the conclusion that it also was a representative of Class C.

Section II.—The Nitrogen Effect with Apples of Classes A and B.

There are in all six records which conform to this type of nitrogen effect, but in this section we shall only consider XIX, XXI, *a*, XXI, *b*, and XXV. postponing to the next section the cases of XI and XXIII, because these two were not in air immediately before exposure to nitrogen, but in other gas mixtures. Their forms, therefore, need more interpretation than those now before us. All these four cases, as their high serial numbers show, came late in the season, and even the earliest, XIX, had begun to turn yellow in cool storage. On reference to fig. 3 in the first paper of this series, which presents a schema of the senescent respiratory drift of the three classes, it will be seen that all these individual apples, whether belonging to Class A or Class B, are well down the descending slopes of the schema. Had the main lines of grouping of the apples been known beforehand, care would have been taken to investigate also in nitrogen the less senescent apples on the ascending limb of the schema.

Our immediate object in this section is to compare these A and B records, determine the common elements in their form, and show how much their appearances contrast with the typical form of Class C. The graphic treatment will be similar and the four records are to be found at the lower part of fig. 1.

We may first consider the records of apple XXI, *a*, and XXI, *b*, as this apple underwent longer examination than any other case. This apple had been observed in air for 50 hours before nitrogen, and the course of the air-line was well established, having fallen in a steepish curve to the value of 12·4 (XXI, *a*). With nitrogen, there is a big immediate rise of CO_2, reaching the top of a hump at the value of 18·2 in 10 hours and then declining in a fluctuating curve, but all the time well above the air-line. At hour 48, air was given again and there is a rapid drop in the record. When, however, the value reached that of the air-line it did not stay there but carried on, giving a marked dip below the air-line values. This fall gradually slackened off and presently ceased, after which the values slowly mounted to the air-line and then continued along it. The drift of the air-line is here well established, as the apple remained in air for a further 140 hours.

Comparing the records in air, on each side of the nitrogen experience, we see that they can be joined up to give one regular continuous air-line drift, and it appears that nitrogen has had no permanent disturbing effect whatever. It was decided, therefore, to give an even longer exposure to nitrogen with this same apple, and record XXI, *b*, which, in the general records, should be directly continuous after XXI, *a*, is shown in fig. 1, alined under XXI, *a*, for comparison. The air value of 9·7, at which nitrogen was given the second time, comes just about a week from the beginning of the XXI, *a*, record. Still we find exactly the same reaction to nitrogen, though the hump is here not so high absolutely. The exhibition of nitrogen lasted 97 hours this time, but there is no trace whatever of the toxic effect of nitrogen found in apple XXIV of Class C.

The long record of XXI, *b*, is instructive as demonstrating that the main drift in nitrogen does continue on as a downward slope which converges on the air-line. The track clearly follows an undulating course, fluctuating on either side of the ideal line that can be drawn smoothly through it. The fluctuations get less and less as the pitch of the line declines. Air was given at hour 335, and the record in air is again of the same type as at the end of XXI, *a*, passing through a dip below the air-line at about 18 hours. The whole experiment had now lasted 17 days, and further exhibition of nitrogen on it could not be tried.

We may now turn to apple XIX, which preceded XXI. The respiration in air was first observed for 45 hours and fell fairly rapidly, reaching 14·2, at which point nitrogen was given. There follows a very pronounced hump, reaching the high level of 21·6 at hour 22 and falling in an undulating track till hour 72, the respiration in nitrogen being still greatly above the air-line. At this point air was given causing a rapid fall, and the record cuts the air-line in 12 hours and then dips below it. The temperature adjustment of the thermostat failed here and the experiment was discontinued, but the record, as far as it goes, has exactly the same features as the records of XXI, *a*, and XXI, *b*. As the record was not followed on to return to an air-line, the exact location of that air-line cannot be established, but reviewing all the evidence its course cannot be far from that drawn in the figure.

The last apple of this class to be examined is XXV, which was definitely selected from the cool store as being the ripest and yellowest apple showing no trace of brown that was present at that date (June 21). Apples of the C class were still greenish, and most of the A apples had rotted, and there is some evidence for regarding XXV as belonging to the B class, which is held to ripen a little slower than A but much faster than C. This case was observed in air for 55 hours, during which time the respiration fell gently to 12·5. Nitrogen was then given and produced the usual quick rise, to a maximum of 15·8 in 7 hours. Then the respiration declined quickly by a slightly wavy record for 48 hours, rapidly converging on the air-line. When air is given the record shows a new feature, in that the CO_2-production first rises to give a small hump and then slopes away slowly to just below the air-line, afterwards rising gradually to it. It is curious to find that the superficial form of the record in air after nitrogen, in this case, more closely resembles its own record in nitrogen after air than any other record of air after nitrogen. Clearly this apple has some special features, and at first it was thought that these might be characters of Class B, but again it is the extreme end of the ripening series and its special features might be the expression of this position: the latter is the view that we adopt when in the next section we come to consider yet another apple of Class B, namely XXIII, which comes just before XXV in the ripening series and provides a transitional form to this extreme, all within the essential limits of the type we are considering.

We may sum up the characteristics of the nitrogen effect with apples of Classes A and B as being, first, that the respiration lies well above the air-line instead of upon it, and, second, that it presents a continuous slow decline. In appearance it may be imagined that the decline, which is faster than that in air, would ultimately bring the nitrogen CO_2 down to equality with the air-line value, such as characterises the C class, but we have not been able to pursue the problem so far for Class A.

The only feature that appears to be common to the two classes is that the first immediate effect of nitrogen is to cause the CO_2-production to rise sharply above the previous level in air. The fact that these two classes should differ in every other particular feature seems to us a striking phenomenon and evidence of a metabolic difference, the general nature of which was only characterised after long analytic study.

On comparing the features of the return to air after nitrogen, it is clear that one essential contrast must exist between the types, arising out of the fact that the A apples have a long way to fall to the air-line while the C apples have been depressed below it, and must rise. Neither of these changes proceeds by what we may call the most direct route. Class C typically shows a sudden transient rise of respiration on return to air, but this subsides again before the final rise to the air-line is achieved. In Class A the rapid initial fall is not arrested when the air-line value is first reached, but sweeps below it in a transient dip from which it rises to attain the air-line finally. With apples of Classes A and B in the most advanced senescent stage, this typical form undergoes a modification, which suggests an affinity with the type of Class C, in that a transient hump of CO_2-production is the initial reaction to air, and this phenomenon is, as it were, superposed on the fall from nitrogen values to the air-line.

Section III.—The Nitrogen Effect after Gas Mixtures other than Air.

With all the nitrogen records considered in the previous sections, the apple was respiring in air up to the change into nitrogen. We have now to consider the two cases in which the apple was in an atmosphere either much richer in oxygen (100 per cent. O_2, apple XXIII) or much poorer in oxygen (5 per cent. O_2, apple XI). Examination of these records will show what effect this difference of antecedent conditions has upon the respiration in the nitrogen period. Both apples were returned to air at the end of the period in nitrogen. In fig. 2 the records of the nitrogen experience of these two apples have been brought together, alined at the transition to nitrogen.

Apple XI—Transition 5 *per cent.* O_2 *to Nitrogen.*—The apple was in air up to hour 24, which locates the first stage of its air-line, but was then treated with 5 per cent. oxygen. The effects of this gas will be dealt with fully in the fourth, paper of this series : it suffices here to observe that the CO_2-production steadily falls in 5 per cent. O_2 till at hour 67 it lies at about 0·7 of the air-line value.

At this hour pure nitrogen is given, and we see the respiration rising steadily, cutting the air-line in about 12 hours and continuing to rise well above the air-line in a way which is characteristic of Class A and not of Class C. The maximum value is not reached till 24 hours, and then there sets in the steady decline of the A type, converging on the air-line. The course of the fall is not well established as owing to failure of clockwork six three-hour readings are merged into one of 18 hours. At hour 110, after 40 hours in nitrogen, air was given, and the course will be commented on shortly.

Apple XXIII—Transition 100 *per cent.* O_2 *to Nitrogen.*—This apple was initially in air for 52 hours, so that its air-line is well defined. At this hour pure oxygen was given, and we see the respiration rising steadily to a level far above the air-line and then maintaining a line 1·40 times the value in air.* At hour 124, nitrogen was given, and in spite of the high air value, the respiration rises, at first still higher, but then declines steadily, as is characteristic of an apple of the A or B class. At hour 174, after 50 hours in nitrogen, it is still markedly above the air-line. At this hour air was given.

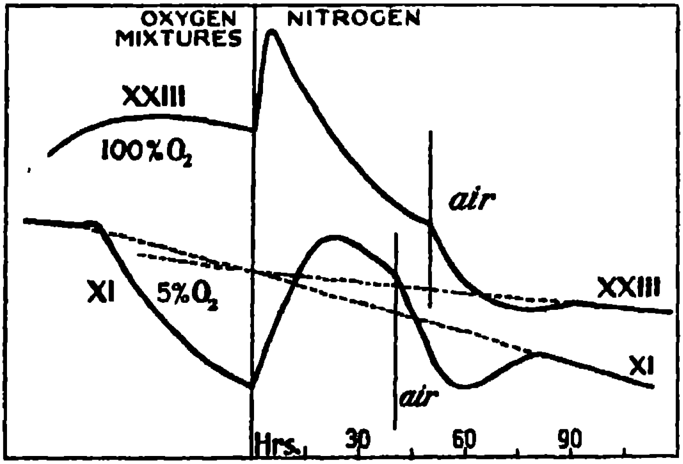

Fig. 2.—Two examples of the nitrogen effect after gases other than air, XXIII from 100 per cent. O_2 and XI from 5 per cent. O_2. Both were returned to air after nitrogen. The whole of the CO_2 record observed is given by the heavy continuous line; the broken lines are the air-lines of the two apples.

From this survey of the records there seems no doubt that apples XI and XXIII both belong to the type of Classes A and B, and neither to Class C.

Transition Nitrogen to Air—Records XI and XXIII.—With apple XI air was substituted for nitrogen at hour 110, and the form of the record seems to be quite typical of Class A. There is an initial big dip below the air-line and presently the record rises again to join the air-line. Apple XXIII, however, has a different form. When given air at hour 174, the respiration falls smoothly and slowly on to the air-line, showing only a slight dip below it. Our interpretation of this form is that it is to be affiliated to that of XXV as an outcome of the late senescent stage. Apples XXI, XXIII, XXV make a good series of drifting forms in this progress, in which XXIII is clearly intermediate between XXI and XXV, for while the latest case, XXV, rises initially before making for the air-line and XXI dips below it initially, we find XXIII drops moderately on coming into air and reaches the air-line slowly, with only a slight dip in the record.

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* The effect of oxygen upon respiration will be expounded in the fourth paper of this series.

Section IV.—The Nitrogen Effect with a Mycelium-Infected Apple.

Apple VIII has a section to itself, because it was the only case in which a patch of mycelium developed on an apple during the course of experimentation. The record of VIII in the Appendix shows that after 60 hours, though still in air, the CO_2-output started to rise at a very rapid rate, quite unlike any other apple of the whole set. The respiration also became very irregular, fluctuating widely from reading to reading, so that the " contour lines " that contain the whole series have to be widely separated. This state of things could only be attributed to fungal development.

Having carried on the record in air for 160 hours, it was thought worth while to give nitrogen, and see what happened when such active mycelium is present. A sweeping fall of respiration sets in from the high level of 22·8 and continues for 36 hours, finishing with a nearly level series at 9·3. After 12 hours more of nitrogen, air was given again at hour 210. On this the respiration rose to a somewhat higher level (probably fluctuating), and after 36 hours fell slowly to the level of 9·3 that it had given in nitrogen. The whole record is so different from others that we can only offer a conjectural interpretation of its features. One thing is clear, namely, that nitrogen cuts out entirely the source of the large irregular output of CO_2 in air. This must turn on the killing of the mycelium by the absence of oxygen, for when air is readmitted there is no recurrence of the high values nor even an upward tendency in the respiration within 80 hours. We can, therefore, use the values after administration of nitrogen as pure apple values, those before being apple *plus* mycelium. The value 9·3 suggests itself as the air-line value for the later part of the record, as it is the final value in air and also holds for the last 12 hours in nitrogen. The air-line for the early part of the experiment lies higher and is assumed to begin from an initial value of 12·2, due allowance having been made for the "initial rise of temperature effect."

All things considered together, we regard this apple VIII as belonging to the same type as VII (the late ripening group C) and characterised by slowly rising respiration initially, under our standard conditions of experimentation. The air-line is accordingly shown rising slowly from 12·2 to 13·4 at hour 60. It may be that this early rise is contributed to by mycelium development, but we assume it due to the apple metabolism up to hour 60. After that hour the air-line of the apple proper has to get to the lower value of 9·3, adopted for the later part. The falling curve forming the middle of the air-line is purely hypothetical, and its considerable fall implies that a number of apple cells are killed to form the brown patch revealed at the finish, in which the mycelium is at work. Were it assumed that mycelium had been at work unobserved from the beginning, then there need not have occurred any rise at all in apple respiration proper, and the necessary fall to the value of 9·3 in the air-line might be quite inconsiderable.

We return to the phenomena in nitrogen and note that this gas was only continued for 48 hours. A glance at the nitrogen record for VII will show that no depression should be produced in this time and the respiration would be expected to lie on the air-line for hours 20 to 48 in nitrogen. This is the state of things we have assumed in the air-line here drawn.

Finally, with regard to the nitrogen after-effect when back in air, we note that in neither of the other cases of group C was air re-admitted before nitrogen depression (the third phase) had set in. We have, then, a new case here, and we may accept this difference as the explanation of the fact that none of the after-values in air lies below the air-line. The fluctuating form of the after-effect may well be similar to those of VII and XXII, though this is not proved, as owing to the stoppage of the clockwork 21 hours of readings were merged into one average value, as seen in the record. Anyhow, there is undoubtedly a sharp rise on passing from nitrogen to air, which is a character of group C. Also, it seems clear that after 70 hours in air, the after-effect of so short an exposure to nitrogen will have completely subsided, so that the value of 9·3 attained may be safely taken to represent the true air-line value.

All the characters of this curious record, VIII, can thus be fairly interpreted in terms of other experiments if it is regarded as being, like VII, a representative of Group C.

Summary of Part I.

The most striking general outcome of our survey of the 10 nitrogen records is that they all belong to one or other of two types, which appear so distinct that there is never any ambiguity about assigning a case to its type. Looking at them from the traditional point of view, which stresses the relation between the magnitudes of CO_2-production in nitrogen and in air, the types seem to have practically nothing in common. It is the purpose of our analytic treatment to determine what is really the common measure of metabolic significance between them.

One most important fact that has been fully established is that long exposures to nitrogen have no permanent disturbing influence upon the metabolism, except in the case of the late Class C apple XXIV, for when returned to air the respiration recovers and returns to the same line of starvation drift that it was travelling along before the nitrogen was given. This line is drawn through all the records as the "respiration air-line," and it clearly expresses some fundamental and dominant ontogenetic drift the march of which is neither accelerated nor retarded to an appreciable extent by exposure to nitrogen, however violent the temporary change of CO_2-production may be. The air-line respiration will provide us with a very stable standard of reference for purposes of analysis.

A second important feature of the nitrogen records is that such striking things happen at the transitions, when the sudden change of gas takes place. Recovery of position on the air-line after nitrogen may take a couple of days and be associated with a temporary phase of very low CO_2-production. Entry into nitrogen, on the contrary, displays only a short transition, and the form is very different with the two types of effects. We hope to elucidate the significance of these transitional forms by the further analysis that we now enter upon.

PART II.—ANALYSIS OF THE NITROGEN EFFECTS AS QUANTITATIVE VARIATIONS OF CO_2-PRODUCTION FROM THE AIR STANDARD.

Our general outlook upon the apples under investigation has been to regard them as individuals which are undergoing a steady metabolic drift in storage. The broad respiratory expression of this drift is that in its early stages the respiration, as measured at 22° C., may keep level for a considerable time, while at later stages the respiration steadily falls off in a starvation curve.

These phenomena were fully set out in the first paper of this series. The only index of metabolic state and drift that was employed in that analysis was the pitch and the form of the "air-line" of respiration. Apples of Class C, which progressed slowly through the ripening drift, provided examples of the level air-line, while those of Classes A and B gave numerous examples of the falling air-line.

The departures of the magnitude of CO_2-production in nitrogen from these air standards might be determined in various ways, and we have to examine all possibilities in a search for fundamental causes. As CO_2-production rates in nitrogen and air tend to be either both at a high level or both at a low level, one obvious enquiry is as to whether there is not some significant *ratio* between the intensities or rates of CO_2-production in the two states. This is the aspect that we may examine first.

Section I.—A Survey of the Ratios of CO_2*-Production in Air and Nitrogen.*

As the air-line of respiration provides us with a stable standard of reference, it is a simple matter to express the CO_2-production in nitrogen or any oxygen mixture as a ratio to the contemporary value on the air-line, which latter gives us the value that would have been produced in air had the apple remained in air all along.

In drawing up our ratios we shall avoid committing ourselves to the use of established terms, like anaerobic and aerobic respiration, and employ the following symbols, which simply refer to actual data of this investigation and the conditions in which they are obtained :—

NR (nitrogen respiration) values signify rates of CO_2-production in pure nitrogen.

OR (oxygen respiration) values signify rates of CO_2-production in air or other oxygen mixtures.

ALR (air-line respiration) values are CO_2-values obtained from the air-line drawn through each record.

TR (total respiration) values are CO_2-values without implication as to their metabolic source.

The expression TR is of value at transitions and in cases that will come up for consideration later, where, in very low oxygen supply, we have mixed effects so that TR is useful for the sum of OR + NR. With regard to the relation between OR and ALR, it is clear that when the apple is in air and the respiration is properly adjusted to air, then OR and ALR are identical. The chief use of ALR values is for the periods when the apple is not in air and the air-line is drawn by interpolation. When an apple is in pure oxygen or 5 per cent. O_2, then the CO_2-production is very different from that in air, and here OR values depart widely from ALR values.

The present section of our analysis is concerned with the variations and drift of the ratio —

$$\frac{\text{NR}}{\text{ALR}} = \frac{\text{CO}_2\text{ value (per 300 grm hours) in nitrogen at any point of time}}{\text{CO}_2\text{ value of air line at the same point of time}}$$

In Table I are set out the values of NR ALR and their ratios which our data provide us with Nine cases out of the 10 described in Part I appear in this table apple VIII which was attacked by mycelium being omitted Against two it is noted that they came into nitrogen from 100 per cent O_2 and 5 per cent O_2 respectively while the other seven were in air before nitrogen The

Table I —Table of NR and ALR Values and their Ratios

Hours in nitrogen		0	10	20	30	40	50	60	70
C VII	NR	(20 4)	16 4	13 7	13 7	13 7	13 4	12 2	11 1
	ALR	13 6	13 7	13 7	13 7	13 7	13 7	13 7	13 7
	NR ALR	1 50	1 20	1 00	1 00	1 00	0 98	0 89	0 81
C XXII	NR	(21 3)	16 9	15 9	15 8	14 5	12 7		
	ALR	16 1	16 0	15 9	15 8	15 7	15 6		
	NR ALR	1 32	1 [illegible]	1 00	1 00	0 92	0 81		
C XXIV	NR	(27 6)	23 0	20 8	17 4	14 5			
	ALR	20 8	20 75	20 7	20 6	20 5			
	NR ALR	1 33	1 11	1 00	0 84	0 70			
B XXIII (after oxygen)	NR	(24 0)	21 3	19 1	16 9	15 8	15 0		
	ALR	13 2	12 9	12 7	12 5	12 3	12 1		
	NR ALR	1 8	1 6	1 52	1 36	1 29	1 23		
B XXV	NR	(16 6)	15 5	14 5	13 4	12 4	11 5		
	ALR	12 5	12 1	11 8	11 5	11 3	11 1		
	NR ALR	1 33	1 28	1 23	1 16	1 10	1 04		
A XIX	NR	(22 0)	20 9	2 0	19 2	18 5	17 9	17 4	17 0
	ALR	14 2	13 9	13 65	13 4	13 2	13 0	12 8	12 7
	NR ALR	1 55	1 50	1 47	1 43	1 40	1 38	1 36	1 34
A XXI *a*	NR	(18 85)	17 8	16 9	16 1	15 4	14 9	[14 45	14 05]
	ALR	12 4	12 1	11 8	11 5	11 3	11 2	11 1	11 0
	NR ALR	1 52	1 47	1 43	1 40	1 36	1 33	[1 30	1 28]
A XXI *b*	NR	(14 65)	13 7	12 9	12 2	11 6	11 1	10 65	10 25
	ALR	9 7	9	9 35	9 2	9 05	8 9	8 8	8 7
	NR ALR	1 51	1 44	1 38	1 32	1 28	1 25	1 21	1 18

							XXI *b*—continued		
Hours in nitrogen		0	10	20	30	40	80	90	100
A XI (after 5 per cent O_2)	NR	10 9	13 5	16 0	15 6	14 5	9 9	9 6	9 35
	ALR	14 9	14 5	14 1	13 7	13 3	8 6	8 5	8 4
	NR/ALR	0 73	0 90	1 13	1 14	1 09	1 15	1 13	1 11

values are set out for points of time, 10 hours apart, from the beginning of nitrogen on to the end of it, which varies from 40 to 100 hours The ALR values have been taken from the smooth ALR line, which figures in each record The courses of observed NR in the original records do not furnish very smooth curves It has been mentioned already that in nitrogen the actual record for apples of Class A fluctuates in a wavy course, and the higher the pitch of the record the more ample are the fluctuations For analysis by ratios we need a smoother line than some records give directly, and the values of NR set out in the table are smoothed values There are, however, only two cases where the smoothing we have adopted alters the observed course in a material way

The cases which have been altered in form are those of XIX and XXI, *a*, the difference between observed and smoothed values being set out in fig 3

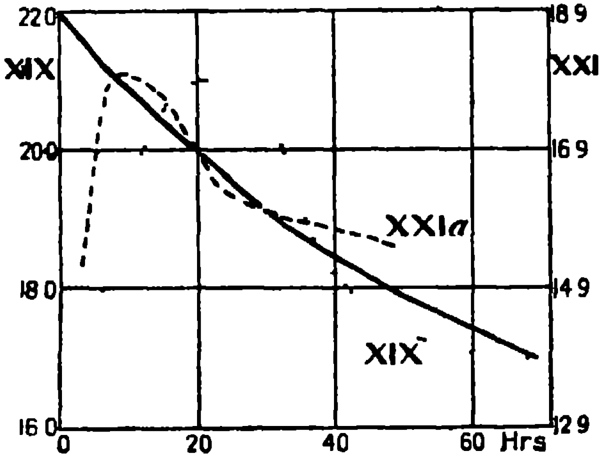

Fig 3 —The smoothed curve of the nitrogen effect adopted for the apples XXI, *a* and XIX for which the fluctuations observed in nitrogen were very great The individual records are given as broken lines, the common smooth form of NR as a heavy continuous curve *The pitch of the two records is different the respective ordinates* being given at the two sides

In this figure the observed points are derived from the full records in the Appendix by taking the middle value between the "contour lines" at the specified hour The assumption started from in smoothing has been that, as these two apples both belong to Class A and are adjacent in date and in condition, they should ideally have a common form of falling NR curve, and that departures from it should be regarded as individual fluctuations The mean form adopted treats the high values of XIX at hours 20 and 30 as being excessive and the low values at hours 40 to 60 as defective With XXI, *a*, on the contrary, hours 40 to 50 give excessive values. In support of the expectation of similarity is the fact that the air-lines of these two apples have identical forms. Adopting the common form of NR curve drawn in this figure and giving it the appropriate pitch for these two apples, we arrive at the smoothed series of values that appear in Table I

The calculated NR/ALR ratios set out in Table 1 are all plotted against time in fig. 4. There is a group of four in the middle of the figure which drift downward in the same general way; these are the apples brought into nitrogen from air, while it is seen that XXIII, which comes from 100 per cent. O_2 starts with an exceptionally high ratio for NR/ALR but falls steeply, till it joins the middle group after hour 30. Apple XI, on the other hand, which comes into nitrogen from 5 per cent. O_2, starts abnormally low and proceeds to give rising ratios, till it also joins the middle group about hour 25 and then falls off in a similar way. Our first survey of ratios establishes a new and important fact, which is, that the previous oxygen experience completely alters the NR/ALR ratio for at least 24 hours after entry into nitrogen, but

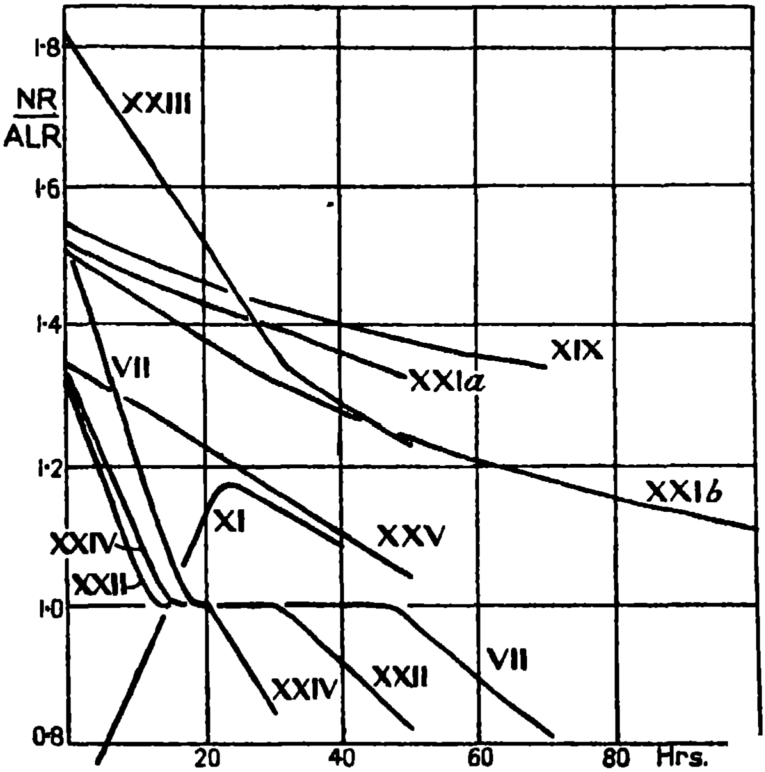

FIG. 4.—A chart of the time drift of the NR/ALR ratios set out in table I. Continuous curves based on the values calculated for 10-hour intervals.

that later on we get ratios that are about the order that we might expect had the apple come from air into nitrogen.

Coming from an oxygen mixture richer than air we get higher ratios than from air, while lower ratios are obtained when the apple has come from the lower concentration of 5 per cent O_2 This we register as a significant principle which in itself is in opposition to the idea of the whole situation being dominated by constant ratio relations Even setting aside these two special cases of XXIII and XI, we find little support for any constancy of ratio The table shows us that if we had to make a general statement for the behaviour of these apples in nitrogen after air, we could only say that the ratios observed ranged between 1 55 and 1 00

There is, however, one set of ratios, which we may call the " initial NR/ALR ratios," which exhibits a certain uniformity It will be seen in the table that we have given in brackets an initial value for the NR series at the zero hour of entry into nitrogen This is arrived at by graphic extrapolation of the observed series of NR values set out in the table When the apple passes into nitrogen from air, the CO_2-production rises considerably and fairly quickly, but it takes some hours to load up the tissues of the apple with the new higher CO_2 content, and it is usually 7 to 10 hours before the rising transition brings the CO_2 escape up to the falling line, on which all subsequent NR values lie The number of points available gives the extrapolation a fairly obvious course, and so we regard these initial NR values as a close measure of the values that would be given if the air supply could be cut off instantaneously and the new higher CO_2 content were to require negligible time for adjustment As these initial values provide in each case the maximal value and maximal ratio for the function NR, they are obviously of considerable metabolic significance

Let us then examine the initial NR/ALR ratios class by class For Class A we have cases XIX XXI, *a*, and XXI, *b* all coming from air to nitrogen, and we find the ratios 1 55, 1 52, 1 51, which from our present point of view we may regard as practically identical. Since we know that XXI, *b* was carried out on the same apple as XXI, *a*, with an interval of 140 hours in air between, it is striking to find the same initial ratio for both This demonstrates that the initial ratio is not a relation which shifts with time for a given apple as the metabolic drift progresses Even though the series of ratios had fallen to 1·33 at the end of XXI, *a*, yet, after recovery of the apple on to its proper air-line, the same initial ratio is given on a second nitrogen treatment, and this, though the absolute pitch of respiration had fallen considerably

Turning to the apples of Class B we have only the case of XXV coming into nitrogen from air Here the initial ratio is 1 33 In Class C we have three cases the very early case of VII gave 1 50, while the two late cases XXII and XXIV give the ratio value of 1 32 and 1 33 It thus appears as if the initial ratios after air group round two distinct values which we may round off as the values of 1 50 and 1 33 All the 1 33 relations observed come in apples at the end of the series such as XXII XXIV XXV while the earlier give 1 50 ratios but as the classes are not uniformly distributed along the series it may yet be held that 1 5 characterises Class A 1 33 Class B while Class C shows 1 5 at the beginning and 1 33 values six months later

When we turn from this evidence of uniformity in initial ratios to consider the falling series of ratios for the successive hours as set out in Table I and fig 4 we find no signs of any simple system The two apples of Class B show a more rapid fall of ratios with time than the three of Class A but even within this last group though the initial ratios are close together at 1 5 the falling series diverge considerably Nor on this extended time scale is it easy to form any conclusion as to the ultimate value of these ratios had each nitrogen experience been kept on for many days longer

We may therefore present our data in another graphic way in which the indefinite extension of the time axis is telescoped Fig 5 enables one to inspect the drift of each type of respiration as well as the ratios between them Here the NR values are plotted against the corresponding ALR values the two axes NR and ALR having their common origin at zero values at the bottom right-hand corner Each pair of values from the table gives a point on this co ordinate system and the points for each individual are connected up with lines which combine them into a drifting series The initial point of each apple is distinguished by a circle

As both forms of respiration are diminishing by starvation we should expect on any simple scheme that each apple would begin somewhere towards the top left hand corner of the figure and drift towards the final extinction of respiration represented by the origin at the lower right hand corner If NR and ALR should maintain a constant ratio throughout the whole progress then the successive points would, of course lie on a straight line passing through the origin A few such constant-ratio lines have been drawn in the figure as construction lines, the constant ratios they stand for are indicated at the left above There are lines for the following ratios 1 00 1 30 1 34 1 52 and 1 55 It is at once clear that the co ordinated values of NR and ALR show no sign of drifting towards zero values in a direct line They move across the figure to the right looking at first sight as if they would in time cut the ALR axis high up and provide the strange situation of considerable ALR values in association with zero values of NR

We may look for a moment at the individual cases in this figure Cases XXI *a*, and XXI, *b* are of great interest because they represent two successive exposures to nitrogen with the same apple Apple XXI, *a*, has its initial fairly towards the left and lying on the 1 5 ratio track With successive hours up to the end of 50 hours the points move diagonally across, almost in a straight course, and reach a value on the 1 34 ratio line During this period the movement to the right indicates a drop of 4 0 (18 9 to 14 9) (see the table), while the movement vertically downwards indicates a drop in ALR of 1 2 (12 4 to 11·2) This is less than one third of the NR drop Between XXI, *a* and XXI, *b*, there intervenes a period of about 140 hours in air, but when we start nitrogen

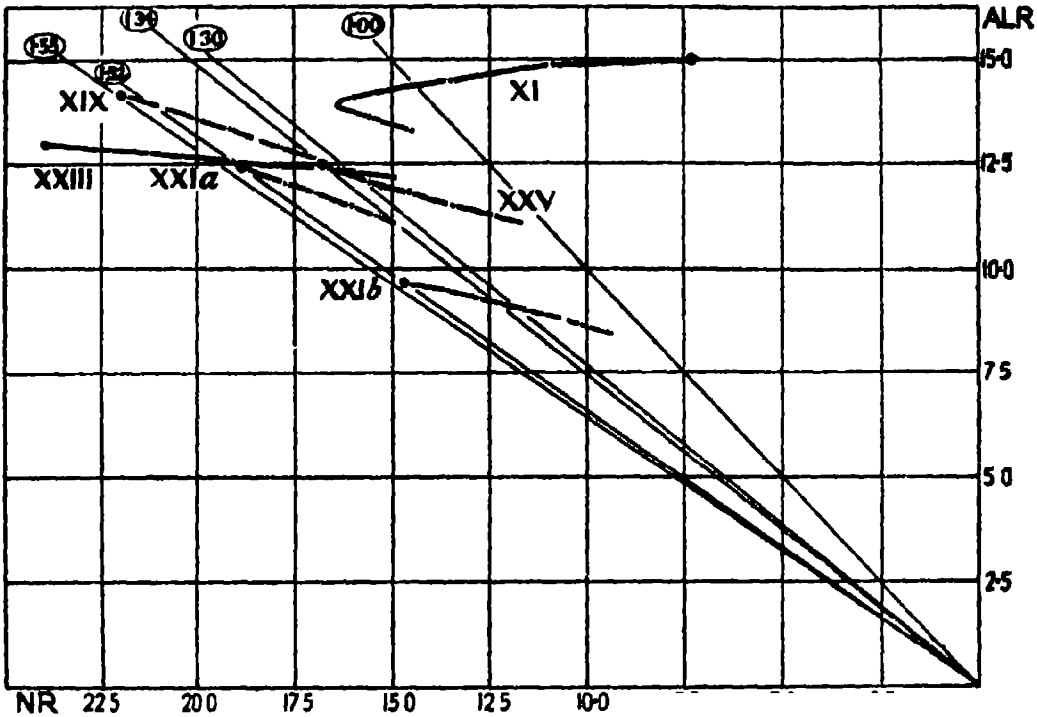

Fig 5 —A chart showing the co ordination of NR and ALR values as they drift with time The axes are values of NR and ALR with a common origin at zero Five construction lines for certain constant ratios for NR/ALR are drawn, and the ratio value for each line is inserted in an oval at the top left hand corner The co ordinated values of NR and ALR for all the Class A and B apples are inserted in the chart by a series of dots for each 10 hour interval as given in Table I The points of each individual apple are linked together by a heavy broken line the initial value being distinguished by a circle

again with the initial point of XXI, *b*, we find that drop of ALR in this air period has been 1·5 (11·2 less 9·7) while the shift to the right, *i.e.*, along the NR axis, has only been 0·2 (14·9 less 14·7). This position allows us to formulate the statement that, when actually in nitrogen, both NR and ALR values fall off (the former about three times as fast), but when in air, though ALR continues to fall off, yet NR hardly falls off at all.

It is therefore made obvious that analysis by series of ratios between NR and the ALR values is not going to clear up the true situation. The one ratio that appears to have a real significance is the 1·5 ratio, which reappears at the initial of XXI, *b*, still the same as the ratio at the initial of XXI, *a*. Though both respiration values at XXI, *b*, are lower absolutely than at the beginning of XXI, *a*, yet the ratio of them is maintained. This suggests a simple form of experimental relation, namely, that if one continued to alternate nitrogen and air during the starvation progress towards zero at the origin, every one of the initial points on passing into nitrogen would lie on the 1·5 ratio line, which makes directly towards the origin. This we may hold fast to, as being a significant fundamental relation.

With regard to the drift of NR and ALR values during the nitrogen experiences of XXI, *b*, we see a repetition of the rapid drift to the right, so that after 50 hours NR has dropped 3·6 (14·7 less 11·1) and ALR 0·8 (9·7 less 8·9), the former 4·5 fold the latter, so that the line of drift is more horizontal than in XXI, *a*. During the further 45 hours in nitrogen the values show signs of working downwards. Possibly this downward drift would become more marked had nitrogen been continued longer, so that presently the value would have got on to the 1·0 ratio construction line. This is merely a formal suggestion at present; we see that the points on the track of XXI, *b*, representing 10-hour periods, are already getting very close together, forecasting perhaps another 100 hours in nitrogen to attain the 1·0 ratio construction line.

Passing now to the other apple of Class A, namely, XIX, which was earlier in chronology than XXI, *a*, and has therefore higher ALR values, we find that it has also higher NR values and that its initial point has again the 1·5 ratio relation, so that by this and by its drift of values while in nitrogen it takes its place on the chart quite as if it were an earlier nitrogen experience of apple XXI.

When we consider the only other case of transference direct from air to nitrogen, that of the Class B apple XXV, we note that its initial ALR has about the same value as XXI, *a*, but its NR value is less, so that the initial point lies on another construction line, that of the 1·33 ratio. Kept in nitrogen, it shows exactly the same type of behaviour as the Class A apples, drifting rapidly to the right, NR having dropped in 50 hours 5·1 (16·6 less 11·5), while ALR has dropped 1·4 (12·5 less 11·1), nearly a fourfold drop in NR. This drift brings it by this short time nearly on to the 1·0 ratio line and it is still drifting rapidly. So that we conclude that though a succession of initials in nitrogen would, for this B apple, fall always on the 1·33 construction line, yet long-continuous nitrogen would probably drift the values across the 1·0 ratio line sooner than in Class A.

The other Class B apple, XXIII, shows the effect of exposure to high oxygen concentration before nitrogen in that the nitrogen initial has a much higher NR value than any other apple, though the ALR value is much lower than XIX and little higher than XXV, the ratio here being as high as 1·82. It recovers from this temporary effect after 25 hours, and its three later values are so placed that they mingle with the points of XIX and XXV.

Apple XI shows the opposite displacement from XXIII. Its initial ALR value is the highest of the lot, being an earlier Class A apple, but as it has been in 5 per cent. oxygen before nitrogen, its early NR values are very low and rising rapidly while ALR falls. These are special transitional values, and the first three points might well have been omitted from this graph. Soon after the fourth point, this transition is over and its maximum NR value of 16·4 is reached. Now at last the record begins to behave like the other cases and shows values drifting across to the right and a little downward. The track is such that if nitrogen had been continued longer the values might soon have drifted on to, and across, the 1·0 ratio construction line. This apple is the only early Class A apple investigated in nitrogen, and its case is difficult because we have no example of this particular metabolic state which had been brought into nitrogen directly from air. We return to this case in Section III of the next paper after further analysis has been carried out.

None of the Class C values has been entered in this figure because it is clear that, since ALR values remain practically constant throughout their records, the series of NR points will be merely a procession across the graph from left to right on one horizontal line, and only the distances between the points, indicating periods of 10 hours, will have any significance. Until we come to investigate apples so early in storage that their ALR line is definitely rising, Class C furnishes the extreme type, giving points on a horizontal line, whereas all the A and B apples lie on a line falling somewhat away to the right. The slopes of these A and B lines are nearly all about parallel, moving 3 to 4 units to the right for a drop of 1 unit vertically, indicating that NR is falling off three or four times as quickly as is ALR.

The absence of any tendency for the series of experimental points in this figure to move towards the common origin during the fairly long periods that the apples were under examination suggests some essential divergence of the course of NR from that of ALR The drift apart of the two values is of the type that would be expected if NR were drifting towards zero at a relatively rapid rate while ALR drifted in that direction at a very much slower rate The striking thing is that recovery of the ALR position is complete on going back to air so this tendency does not persist in air after nitrogen

If an apple after being kept in nitrogen for 100 hours could not be brought back to its ALR values by subsequent air but was found to be permanently depressed then it would be assumed that some toxic effect had inactivated the machinery but of this there is no sign and we regard the depression as metabolic The only possible type of toxic effect that would fit the facts would be an auto depression of NR by some product such as alcohol or acetaldehyde that accumulated in the tissues combined with the absence of depression of the ALR values subsequently Instead of complete absence of depression of ALR subsequent to NR it would be possible to hold that the toxic substance was rapidly oxidised to an innocuous one in the early hours of the return of the oxygen supply

In this connection we have calculated the expected alcohol content of the apple tissues at the end of several of the nitrogen experiences on the basis that 1 molecule of alcohol is produced in association with each molecule of CO_2 This works out at 1 045 mgs alcohol added to the tissues for each 1 0 mg CO_2 escaping during the progress of NR In the 70 hours of XIX 439 mgs CO_2 escape and therefore 457 mgs alcohol are accumulated giving a concentration in the tissues of 0 45 per cent In the 100 hours of XXI *b* the totals per 100 grms of apple are respectively 369 mgs CO_2 and 384 mgs alcohol giving a concentration of 0 38 per cent alcohol Direct experiments have yet to be tried upon the effects of small additions of alcohol No allowance for any such depressant effect has been made in the present survey

By our inspection of NR/ALR ratios we have been led to attribute a certain amount of individuality to NR behaviour by which it departs from ALR behaviour We have also established the definiteness of initial NR/ALR ratios after air and further that quite different ratios are obtained when the apple has been brought from higher or lower concentrations of oxygen We may now take up another line of analysis

Section II Analysis of the Relation of ALR and NR Drift by Difference of Values

The uniformity underlying the relation of a pair of falling curves if not to be found in their ratios might possibly reside in their arithmetical differences and when we turn to investigate this aspect of the pairs of ALR NR curves we come across a significant relation The only region where we have several suitable cases available for comparison is the group of three records XIX XXI *a* XXI *b* all of which are Class A and closely adjacent in date and condition

In all these cases NR is greater than ALR and the differences NR less ALR as derived from the values set out in Table I p 461 are brought together in the upper part of Table II In the column for zero hour where the ratio NR/ALR is for Class A about 1 5 we find a decreasing set of differences in the serial order of the cases each difference being of course half ALR Similarly the differences decrease in the column for each other hour Inspection of the whole set brings out that all the series of differences run a parallel course The series for XXI *a* is always 1 3 to 1 2 below that for XIX and the series for XXI *b* is always 2 8 to 2 75 below XIX The sets of values are characterised then by constant differences * The record for XXI *a* actually

Table II —Table of Differences NR less ALR
(For Values of NR and ALR see Table I p 461)

Hours in nitrogen		0	10	20	30	40	50	60	70
Class A	XIX	7 8	7 0	6 35	5 8	5 3	4 9	4 6	4 3
	XXI *a*	6 5	5 7	5 1	4 6	4 1	3 7	[3 35]	[3 05]
	XXI *b*	5 0	4 2	3 55	3 0	2 55	2 2	1 85	1 55
Class B	XXIII	10 8	8 4	6 4	4 4	3 5	2 9		
	XXV	4 1	3 4	2	1 9	1 1	0 4		
Class C	VII	6 8	2	0 6	0 6	0 0	0 3	1 3	−2 6
	XXII	5 2	0 3	0	1 0	1 2	2 9		
	XXIV	6 8	2 2	0 1	3 2	6 0			
Class A	XI	4 0	−1 0	+1 9	1 9	1 2			

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* It may not escape the reader's notice that contribution has already been made to the parallelism of XIX and XXI *a* by the procedure adopted for smoothing the NR curves for these two cases carried out in fig 3 Had not the ALR values also run a parallel course for the two apples then the parallelism of the differences of the pair would not have resulted so that the two results do give more than a single measure of support to the independent case of XXI *b*

stopped at hour 50, and the two end values in brackets are additions calculated on the basis of constant differences so that we may obtain three sets of 70 hours duration for further examination

In fig 6 we present all the NR curves in terms of their excess values over their companion ALR curves Those of Class A now under discussion are there grouped at the bottom It is clear that the more starved the apple becomes giving lower ALR values the smaller will be the initial excess of NR if the NR/ALR ratio remains constant Since the NR curves are all parallel the smaller also will be the excess of NR at each successive hour To the three upper observed curves of this group we have added an imaginary lower curve also running a parallel course This gives the curve of NR that

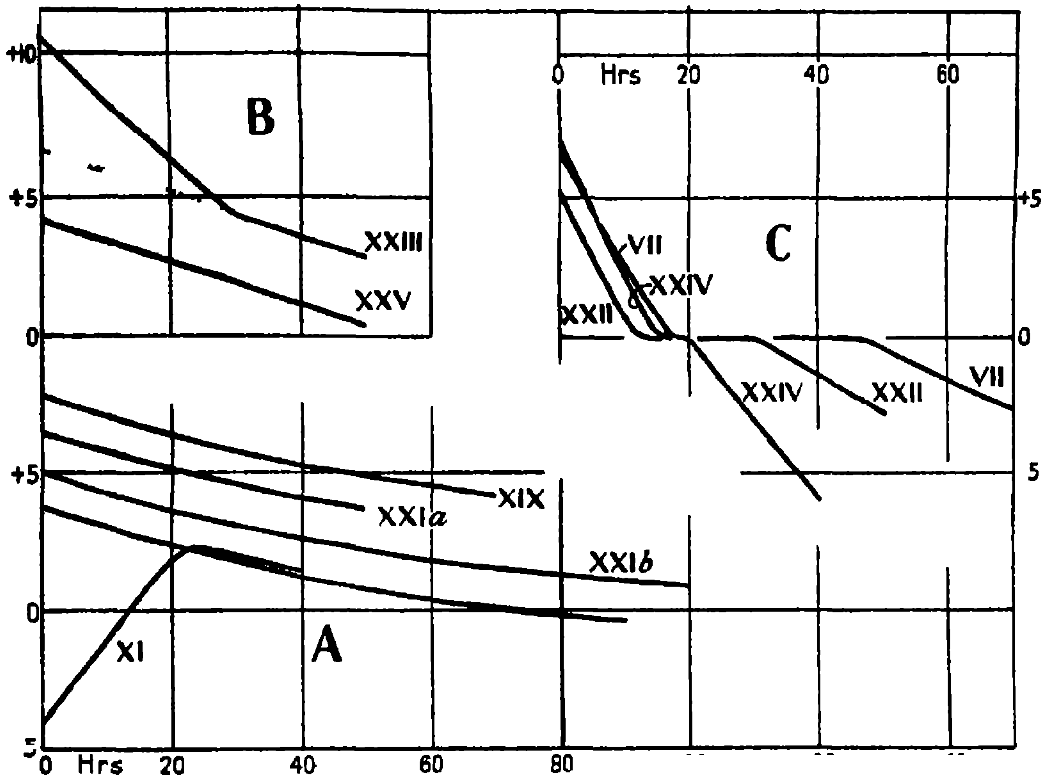


FIG 6—A chart of the drift of differences NR less ALR with time in nitrogen The three classes of apples A B C are given in separate groups In Class A beside the four numbered curves which run a parallel course an additional curve has been constructed on the same basis but of lower initial This unnumbered curve cuts the air line at hour 70 In Class B a dotted addition has been made to the observed curve of XXIII which came to nitrogen from pure oxygen This constitutes a suggestion of the form that might be expected had XXIII been brought into nitrogen from air instead of oxygen The group of Class C curves is described on p 475

we should expect on these principles when ALR falls to 6 9, and therefore the excess NR over ALR becomes initially half this value, 3 45 This curve has been selected as one that would just cut across the ALR curve at hour 70 After that hour the NR values will be below the ALR

How much further all these NR curves would drop after hour 70 cannot yet be safely predicted The curve for XXI, *b* was followed on to 100 hours and continued to fall and approach the ALR value Graphed on this scale it looks as if it might flatten out and never reach ALR but plotting on a more condensed scale suggests arrival there in 170 to 200 hours

In order to present the relation that we have just made out for this group of curves in a more realistic setting we have drawn up fig 7, in which the three cases examined are set out on one time axis As XXI, *a* and XXI, *b*, were carried out on the same apple with a known time in air between them these two cases can be accurately spaced on the time axis The case of XIX preceded XXI but as it was a separate individual the two time scales cannot be combined into one accurately We have therefore located XIX in front of XXI *a* to such an extent as will allow its air line to run on into the line of XXI *a* in a natural falling curve just as if all three cases had been carried out on one apple The general disposition of the parts of this figure can be checked on the detailed records in the Appendix to this paper

The heavy line right through the figure gives the absolute ALR values and the three falling NR curves are also marked in heavy lines As construction lines we have connected up the three initial NR values by what we may call an ' initial nitrogen line " This line could have been drawn by eye well enough but the values were calculated from the known ALR values in the three cases by using the factors established for the three actual initial NR values, these factors being as Table I shows 1 55 ALR for XIX 1 52 ALR for XXI, *a* and 1 51 ALR for XXI, *b* We have added further a line connecting up the NR values observed at hour 30 for the three cases and another line for the values at hour 70 We see that the " 30 hour nitrogen line " and the " 70 hour nitrogen line ' are practically parallel to the " initial nitrogen line '

The relation of this sytem of nitrogen lines to the ALR line now becomes clear Since the initial nitrogen line has an approximately constant ratio (1 5) to the ALR line, then the other NR lines parallel to it cannot have a constant ratio to this same line They must tend to cut the ALR line in serial order at points of time which are earlier the lower the absolute value of ALR

The lowest NR line we have drawn is the " 70-hour line ", the 100-hour line would be lower still but only XXI, *b*, provides us with a point for this line.

Uncertainty as to the form of the continuation of the NR lines after hour 100 prevents us from completing the schema, but we have suggested that these lines continue as falling straight lines at the slope that hours 90-100 indicate.

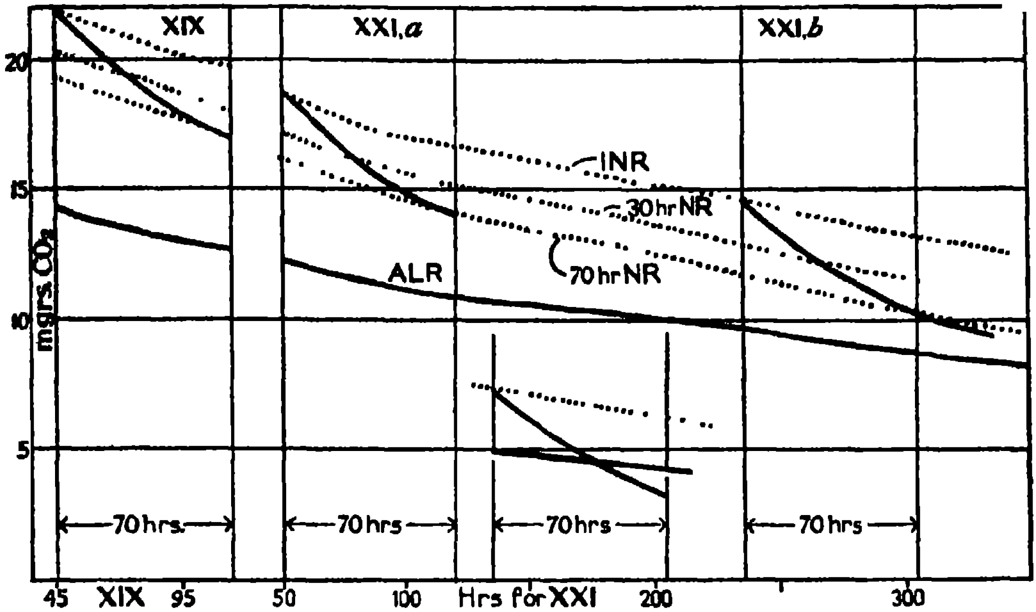


FIG 7.—A construction bringing together the two nitrogen effects of apple XXI, *a* and *b*, and that of XIX for comparison of the relation of drift of NR to drift ALR. The two parts of XXI are correctly spaced on the actual time axis of the record, which is given below, but the case of XIX is arbitrarily placed on this time axis, so that its ALR line runs on smoothly into that of XXI. The heavy continuous curves above represent the course of downward drift of NR in the three cases between vertical lines marking off the first 70 hours in nitrogen. With XXI, *b*, the NR curve was observed longer, on to 96 hours but with XXI, *a*, the actual observed period was only 50 hours, the values for hours 60 and 70 being calculated as shown in Table II.

Three construction dotted lines are added to the nitrogen part of the record. These join up, for the three cases (1) the initial NR values at zero hour, (2) the values at 30 hours in nitrogen, and (3) the values at 70 hours in nitrogen. These three nitrogen lines are practically parallel. It is brought out in this way that the lower the absolute pitch of NR initially the sooner will the continuation of the NR line make contact with the ALR line.

At the bottom of the figure in the middle an imaginary case has been drawn up, where ALR is only 5·0 when nitrogen is given. On adding to this an NR curve, constructed on the principles set out in the text, we find that NR cuts ALR as early as hour 40.

As an inset at the bottom of the figure we have added an imaginary case constructed on the principles set out above. Here ALR is supposed to have fallen as low as 5·0, and the initial NR will therefore be 5·0 × 1·5. The initial NR line is drawn at this ratio to ALR and the NR sequence is drawn falling away by the same series of values as in the earlier cases As a result it is clear that in this extreme case the NR line cuts across ALR as early as hour 42, and thenceforward diverges from it below

By this survey of NR and ALR relations we have established a new and significant principle, which is that the grade of starvation is the thing that determines whether the course of NR, when the initial hours are passed, lies well above the air respiration or close to it, or mostly below the air line All these states might possibly be produced in one individual apple, allowing, of course, for the fundamental relation in Class A apples, that the initial NR is 1·5 times the contemporary ALR value This point of view stresses still further the part played by carbohydrate metabolism in determining relations which might *a priori* have been attributed to oxidation processes

Before going further into this aspect of respiration we have yet to inspect our other NR curves and see what indications of regularity based on values of NR less ALR they may present There await us the two cases of Class B XXIII and XXV, the three cases of Class C, VII, XXII and XXIV and the single early case of Class A represented by XI In Table II the values are set out and they are presented graphically in fig 6, Groups B and C

We may look first at the two cases of Class B Apple XXV brought from air to nitrogen distinguished itself from Class A by showing a steep and rectilinear fall of NR/ALR ratios The values of excess NR over ALR also fall off steeply in a fairly straight line, so that in 50 hours, when nitrogen was stopped, the difference had nearly become zero The initial excess of NR is 4 1, giving an NR/ALR ratio of 1 32 instead of the 1 5 ratio of Class A There are thus three minor points distinguishing XXV from the group XIX–XXI, *b*, namely, the steeper fall, the linear course, the early prospective crossing of the ALR line, to add to the different initial ratio

Grouped with XXV in fig 6 there is also the curve of values of differences for XXIII, another apple of Class B As this apple was in pure oxygen before nitrogen, its OR value was much above the ALR, and the effect of this previous history was to give a very high initial NR with an ALR ratio of 1 82 The excess over ALR is initially 10 8 and the values fall off very steeply in a straight line till hour 30, after which the fall continues much less steeply on to hour 50, when the nitrogen was ended In this last part the line of XXIII runs about parallel to that of XXV, but in the first 30 hours the high values are due to the previous exposure to oxygen We can imagine that if XXIII had come into nitrogen from air, the series of NR—ALR differences would have run parallel to that of XXV all along, and a dotted line has been drawn in the figure for hours 0 to 30 on that assumption. This indicates for this hypothetical treatment of XXIII an initial NR − ALR difference of 6·6. The recorded ALR value in Table II is 13·2, so there results on these lines an NR/ALR ratio of 1·50 for this apple going into nitrogen from air. For the companion apple, XXV, of this class the ratio was shown to be 1·33, so it is left uncertain as to whether this reconstruction is valid as a support of parallelism of difference values. We have no experimental test case to guide us.

Turning to apple XI of Class A we have here the opposite distortion from XXIII in that this apple came into nitrogen from 5 per cent. O_2, which had lowered its respiration, and the NR values for some hours are below the ALR line. They rise fairly fast and are well in excess of it by hour 20. After a maximum at hour 25 the values fall off, till hour 40, when, too soon for the full information which we now desire, nitrogen was changed for air. The values in Table II are added to fig. 6, Group A, showing that after hour 25 we get the falling curve characteristic of Class A and also lying parallel with the rest of the class. If this parallelism between hours 25 and 40 is carried back to zero hour, we get the line, figured, but not numbered, which we might expect to be the course followed had apple XI been in air before its nitrogen experience. The initial value of NR excess over ALR indicated by this graphic construction is about 3·7, while the ALR value has been taken as 14·9. As in the case of XXIII, this graphic method does not give a concordant ratio, indicating NR/ALR = 18·6/14·9 = 1·25.

In no other case have we met a ratio that was not close to either 1·50 or 1·33. We must leave this problem open, after recalling that XI is the only early apple examined in nitrogen for Classes A and B, and that special changes of organisation-resistance are still proceeding in this early state, on the lines set out fully in the first paper.

Apples of Class C.—Finally, we have to survey the special class, C, consisting of apples which show striking characteristics associated with their extremely slow rate of ontogenetic development. The values for the excess NR over ALR are set out in fig. 6, Group C. In appearance, this set is widely different from the other two sets, as each case is built up of three jointed linear courses, but nevertheless these lines run parallel for the three different apples The initial excess is high, but then the ALR values are high in these cases. Inspection of the values in the tables will show that there are here two cases of initial ratio = 1·33 and one of 1·55, while in regard to the NR − ALR differences there are two cases of 6·8 and one of 5·2. In spite of this rather mixed state it is clear that the lines are generally parallel in the figure To this falling phase succeeds the level phase where NR/ALR = 1 0 and here parallelism becomes identity of course In the third phase VII and XXII show also parallel movement while XXIV drops somewhat more steeply In this last apple there is probably a toxic factor for the apple succumbed completely with continuation of the nitrogen

Summary of Part II

The main conclusion that we draw from this survey of NR and ALR drifts is that the metabolic factors that determine the progress with time are different for the two cases There is a close correlation of the initial magnitude of NR with ALR but after that the NR values follow a course of their own For full information about this course we should have to study prolonged starvation drifts in nitrogen in the way we have studied starvation in air We do not yet know how far this study could be carried with Class A and B apples without the interference of toxic effects One thing is clear namely that the grade of starvation in air determines whether the NR values lie above the ALR for hundreds of hours or only for a few tens of hours

All these considerations lead us to take up the definite position that the difference between the air condition and the nitrogen condition is something other than the difference between CO produced by oxidation of sugar and CO_2 produced by splitting of sugar The simple ratio relations which might govern a situation determined wholly by oxidation are not found to be applicable We conclude that a consideration of the carbohydrate metabolism which is antecedent to respiration and supplies the substrate for this catabolic complex must be brought into our field of enquiry

This extension of the analysis is presented by Dr F F Blackman in the next paper of this series

APPENDIX

The first four papers of this series of analytic studies deal with various aspects of the respiration phenomena observed in the prolonged investigation of a single set of apples from cool storage For convenience of reference the primary data of the whole set are collected here as an appendix to this second paper As more than a thousand estimations of CO_2 production were carried out for this work the actual values are not printed in tables but are only presented as graphic records of the drift of respiration with time and various concentrations of oxygen

This appendix contains first a table of characteristic attributes of each of the 21 apples, then some general notes on the graphic charts followed by special notes on the individual records and finally a series of charts in which are reproduced the graphic records of the respiration of the individual apples

Notes to Table Each apple that was investigated was assigned a number and is identified by its roman numeral throughout these papers These numbers run in chronological order from V to XXV but as the respiration apparatus was a double one two apples were generally under investigation at once The dates in column 2 are a guide to this coupling and often the two simultaneous records are *presented in one chart* The colours of the apples at the date of column 2 when brought from cool storage to the respiration apparatus are given in column 3 and serve as an index of their metabolic drift towards ripeness

In the table the apples are not arranged in one chronological sequence but are segregated into three groups representing the three physiological classes suggested in the first paper Class A ripens fast Class C very slowly and Class B at an intermediate rate

Column 4 contains the fresh weight of the apple when the record starts The duration of each observed record is given in hours in column 5 At the end of the record the apple was weighed again and the loss recorded is expressed in column 6 as the loss per 10 days per cent of the initial weight All respiration values are referred to 100 grms of *initial* weight Correction of respiration values to the falling weight through the record has not been carried out such formal correction would not seriously alter any of the significant values and it is further open to objections on physiological grounds

Owing to rise of temperature from 2 5 C to 22° C the initial course of CO_2 production has a complex rising form and the true initial respiration value for 22° C can only be got indirectly by extrapolation back to zero hour of the

Table of Characteristics of Apples

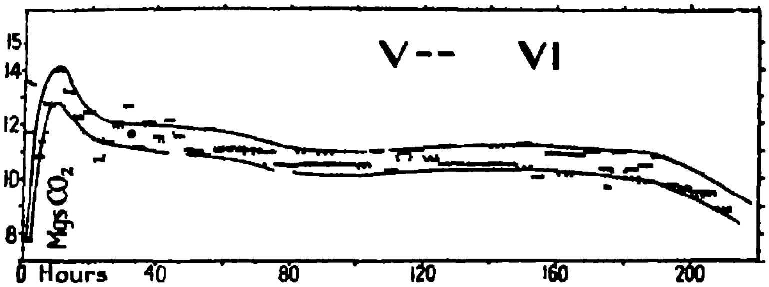
1 Serial numbers	2 Date of record	3 Colour of apple	4 Initial weight	5 Duration of record	6 Loss of weight	7 Air line initial	8 Slope initial	9 Hours to inflexion	10 Hours to maximum	11 Value at inflexion	12 Air line type	13 Air value at 100 hours	14 Fall in 100 hours	15 Gas treatment	16 Page for record
Apples assigned to Class A															
														Per cent	
V	10 11 20	Green	174 2	210	0 77	11 75	13 6	32	10	11 6	II *a*	10 5	1 25	—	480
VI	10 11 20	,,	192 0	210	0 87	11 75	13 6	32	10	11 6	II *a*	10 5	1 25	—	480
IX	15 12.20	,,	126 5	140	1 32	14 0	15 6	25	10	13 9	II, *a*	12 5	1 5	5	481
X	15 12 20	,,	144 0	140	1 73	14 0	15 6	25	10	13 9	II, *a*	12 5	1 5	—	481
XI	11 1 21	,,	127 4	170	1 57	16 8	18 1	20	10	16 8	II, *a*	13 6	3 2	5, N	482
XII	11 1 21	Less green	153 3	110	1 70	16 8	18 1	20	10	16 8	II *a*	15 1	1 7	—	481
XV	10 2 21	Green	156 8	240	—	20 5	—	—	—	—	II, *b*	16 2	4 3	5	483
XVI	10 2 21	,	183 0	240	—	19 5	—	—	—	—	II *b*	16 2	3 3	3	483
XVII	2 3 21	,	132 0	220	1 52	20 6	22 2	22	9	19 6	II *b*	17 1	3 5	5, 7, 9	483
XIX	11 4 21	Yellow	162 6	150	1 84	16 2	19 0	38	11	14 4	III, *a*	13 0	3 2	N	484
XX	30 4 21	,,	141 2	300	1 41	14 3	16 3	29	11	13 1	III, *a*	11 2	3 1	5, 7, 9	485
XXI	30 4 21	,,	145 0	440	1 34	14 3	16 2	29	10	13 0	III, *a*	11 1	3 2	N, N	485
Apples assigned to Class B															
XIII	24 1 21	Green	139 5	200	1 60	14 2	15 4	16	8	13 5	II, *b*	10 5	3 7	3, 5	482
XIV	24 1 21	,,	149 0	200	2 09	14 6	16 5	27	10	13 5	II, *b*	11 3	3 3	3	482
XVIII	2 3 21	,,	138 5	230	1 66	17 7	19 6	30	8	17 3	II, *b*	16 3	1 3	—	484
XXIII	28 5 21	Green yellow	126 1	240	1 67	17 6	19 1	19	13	16 7	III, *b*	13 6	4 0	100, N	486
XXV	21 6 21	Golden	126 7	186	1 50	14 7	15 9	18	9	13 7	III, *b*	11 1	3 6	N	487
Apples assigned to Class C															
VII	26 11 20	Green	134 0	320	0 99	12 8	14 25	24	9	12 9	I, *a*	13 75	0	N	490
VIII	26 11 20	,,	147 0	300	1 01	12 2	13 5	23	8	12 6	I, *a*	—	0	N	491
XXII	28 5 21	Green yellow	145 8	256	2 03	17 3	18 6	23	13	17 6	I, *b*	17 7	0	N	486
XXIV	21 6 21	Yellow green	138 3	230	1 81	18 5	20 1	29	11	18 8	I, *b*	20 9	0	N	487

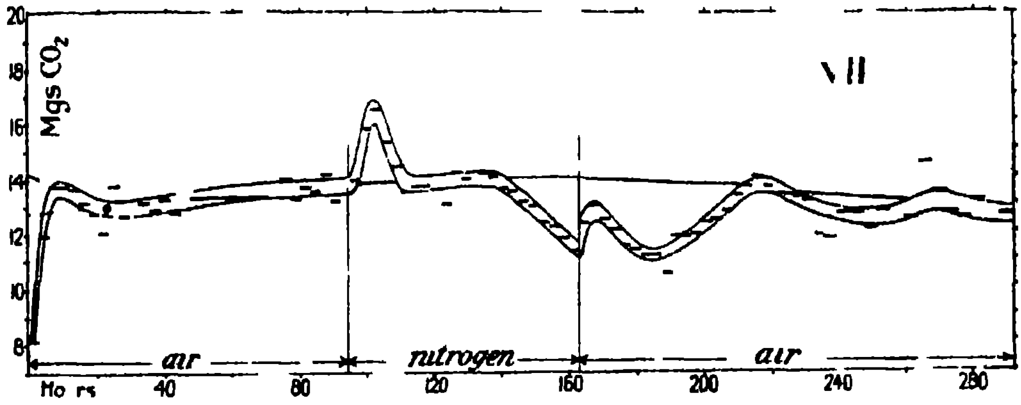
record. The procedure for arriving at the theoretical initial values of CO_2-production is fully discussed in Paper I: the values finally adopted are set out in column 7, expressed as mgrms. CO_2 per 100 grms. fresh weight per 3 hours. This value serves as starting point for the "air line" of respiration which figures conspicuously in the records. The values of air-line respiration 100 hours later are given in column 13 and the drop in value appears in column 14, being the value of 13 less 7. The apples of Class C are characterised by showing no fall of the air-line within 100 hours.

The initial CO_2 values set out in column 8 are the points at which a certain construction line would cut the ordinate axis at zero hour. This line gives the slope of the falling excess CO_2-production due to the initial heating up disturbance. A short length of this line is shown in each chart. Continued downward it would intersect the air-line at the "inflexion" point, marked on the charts by a circle. The time in hours before this point is reached is set out in column 9; and the time to reach the actually observed maximum of CO_2-production on the way to the inflexion point is given in column 10. The CO_2 value at the inflexion point is given in column 11. This value constitutes the earliest observed respiration value on the true air-line, and lies between the values in column 7 and column 13. The types of air-line drift are studied in Paper I and serve to characterise the three physiological classes. The entries in column 12 refer to the classification in Paper I, p. 426.

Lastly, in column 15 of the table are entered the various gas treatments other than air to which the apples were subjected. N indicates pure nitrogen, and the percentage numbers refer to oxygen concentration.

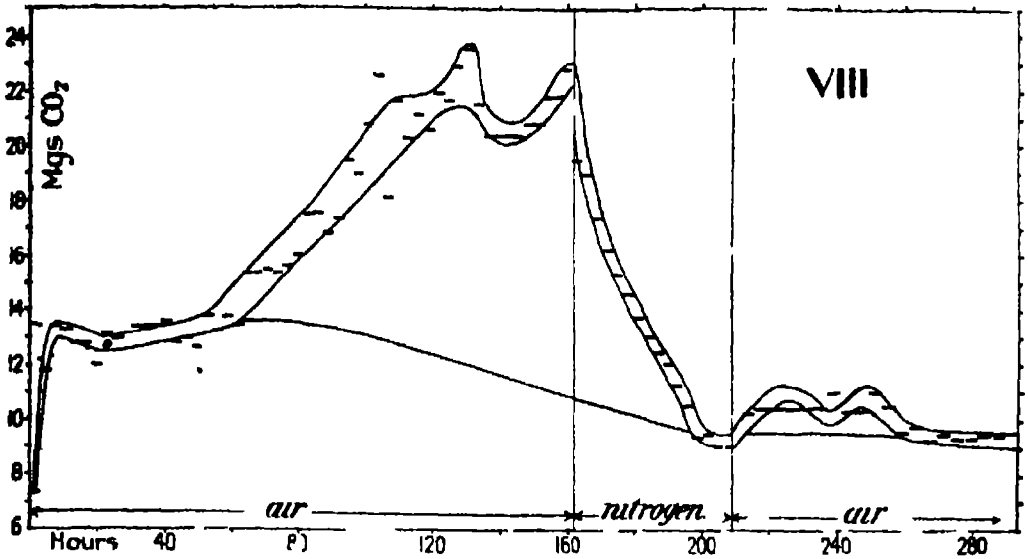
General Notes on the Charts - There are 17 charts, containing in all 22 records, for the 21 apples numbered V to XXV. Apple XXI was observed for a very long period and its record is divided into two portions, XXI, *a* and XXI, *b*. All the charts are presented on a uniform scale as time drifts; the ordinate values indicate mgrms. CO_2 produced per 100 grms. fresh initial weight of apple per 3 hours. The individual readings are all of 3 hours' duration and usually appear as heavy lines of this length in the charts. When two records of different individuals, observed simultaneously, appear in one chart, one of them is distinguished by a row of three heavy dots for each reading instead of a continuous line. These signs are also set against the roman numerals for identification of the two records in the one chart. The drifting series of readings which constitute the "CO_2-record" right through the chart show nearly always the same amount of fluctuation about an imaginary mean line. Such a smooth mean line has not been drawn in the CO_2 records, but instead

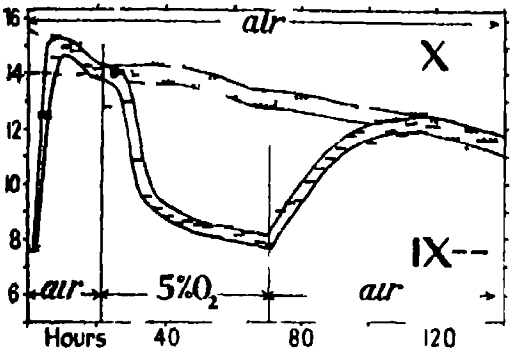


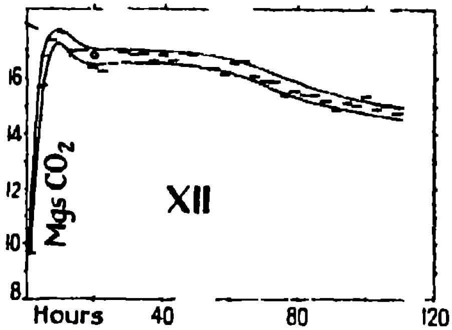


we have drawn a pair of contour lines marking the upper and lower limits of the fluctuation of the CO_2 record When an exact respiration value is needed for our arguments the value midway between the contour lines has been adopted There is obviously often some ambiguity about the location of the contour lines and the mean values the precise turn of form that we have given to the records in various places is the outcome of a good deal of comparison and analysis It is sometimes a matter of choice of interpretation rather than of proof of the relation put forward

Every record starts with a longish period in air and nearly all end with a period in air so that the respiration drift in air may serve as a standard of reference Only five of the apples remained in air all the time the others were exposed to nitrogen or various concentrations of oxygen in the middle parts of the record In column 15 of the table are set out their individual experiences and in each chart there is a horizontal line indicating the sequence and duration of the various gas mixtures In these gas mixtures the CO_2 production may depart widely from that in air and the CO_2 record is drawn everywhere as a series of 3 hour readings enclosed in double contour lines these contour lines are joined up with the air record so as to bring out the transitional course on passing from one gas to another







When the apple is not in air the record contains an additional line which is interpolated smoothly between the parts actually in air This single line is in direct continuity with the imagined mean line between the contour lines in air This track represents the course that the respiration of that particular apple would have followed had it been kept in air throughout the experiment We thus get what we call the air line of respiration ALR right through the record, and this serves as an invaluable standard of reference in all these analytic studies The use of this standard is discussed in the text at various places

At the beginning of each record the course of respiration is much disturbed by the rapid rise of temperature from 2 5° C of cool storage to 22° C of the respiration apparatus Special study has been given to the initial rise of temperature effect" in the first paper of this series Various construction symbols are inserted in the charts to characterise the magnitude of this effect
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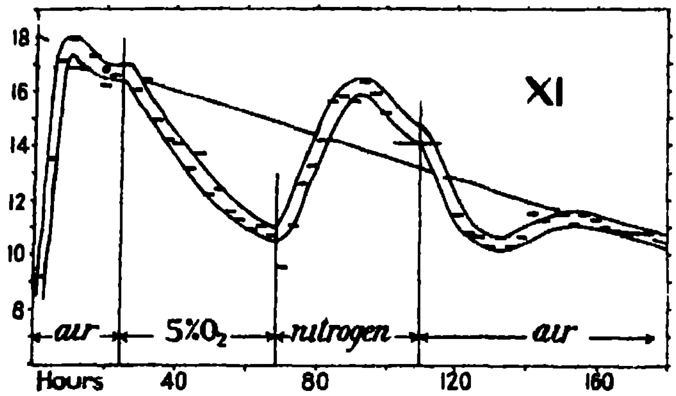
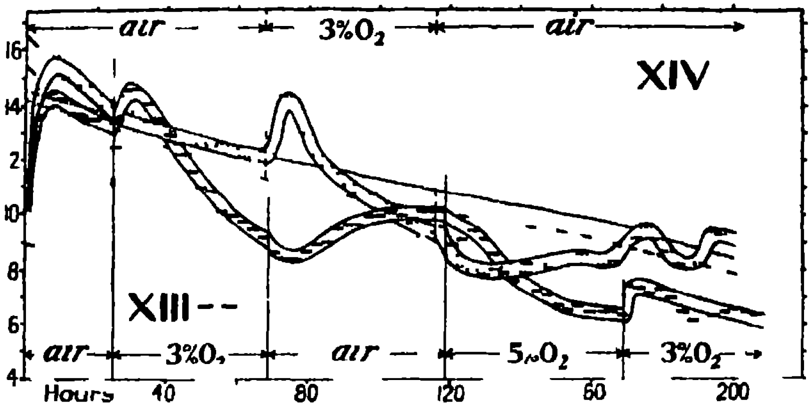
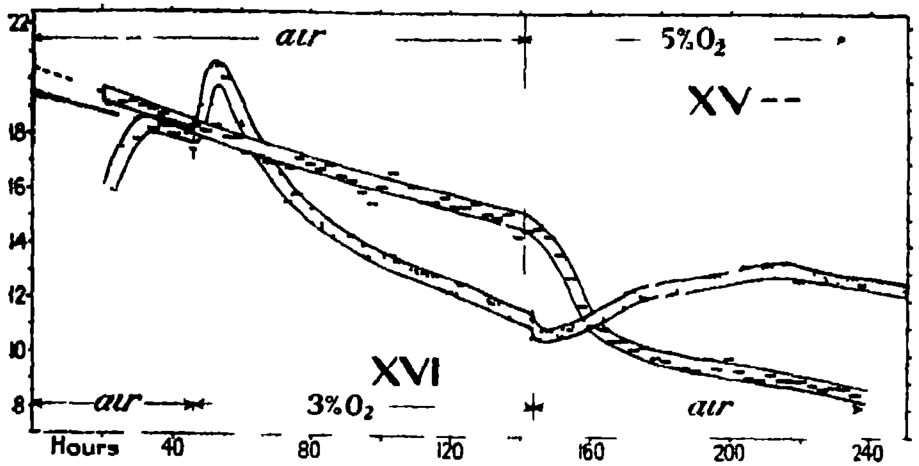
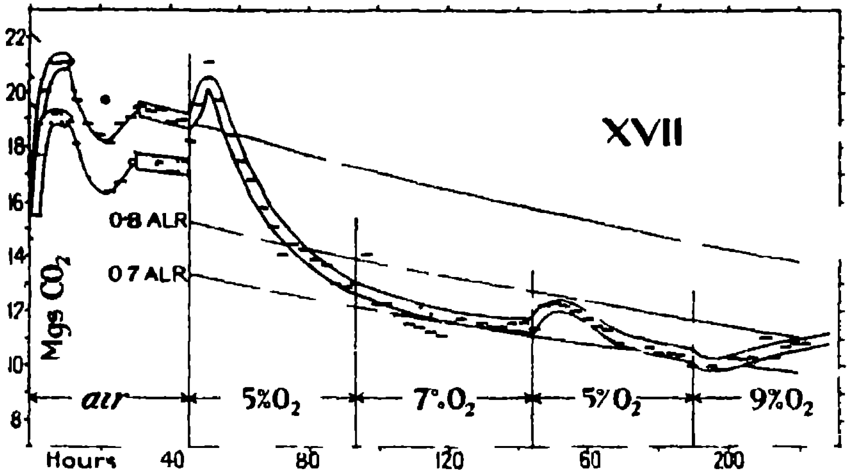




The circle between the contour lines locates the so called inflexion point which marks the end of the initial disturbance and is thus the first observed point on the true air line of the record. Carried back from this point is a broken line extrapolated to cut the ordinate axis, and giving the theoretical beginning of the air line. The value at zero hour gives the true *initial* respiration value (see column 7 of the table). Actually the CO_2 production has shot above this line temporarily and has then sloped down to the inflexion point. A short heavy line pointing down to the inflexion point is drawn for a few hours from zero hour in each record so that the initial value indicated by extrapolation of this slope is also brought out. The isolated heavy dot some way beyond and below the circle put in each chart serves merely to define more accurately the slope of this line through the inflexion point. The time duration of the phases of this initial temperature disturbance are set out in the columns of the table.

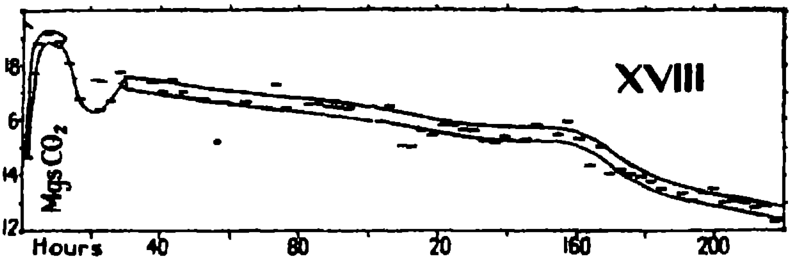
The symbols ALR, OR and NR, standing respectively for air line respiration, oxygen respiration and nitrogen respiration, are defined more closely on p. 460 of Paper II.

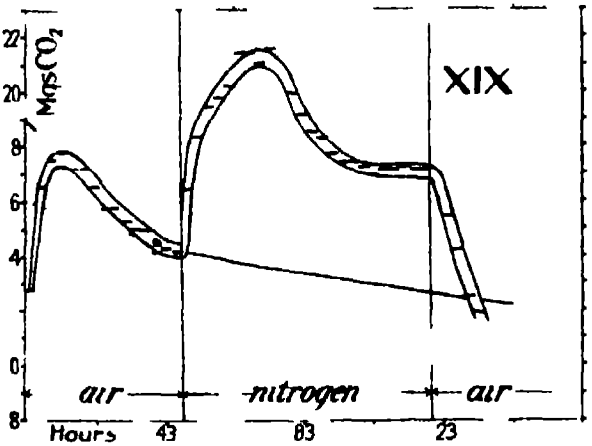




Special Notes on the Records

Records V and VI (see p 480) —This was the first observation carried out and the two apples were kept in air throughout In the chart the data for each apple are entered separately, V as lines and VI as sets of three dots but each record is not provided with its own pair of contour lines Instead, two more widely separated contour lines enclose the double record, and the mean line between them would present the average air line of the two apples The form of the air line beyond the inflexion point circle is well founded and drops very little until hour 180 It will be noted that scattered readings occur well outside the contour lines, but these are not taken any account of in our presentation of the records The dotted line for the early part marks the ideal air line, giving its initial and course, while the actual CO_2-record shoots temporarily

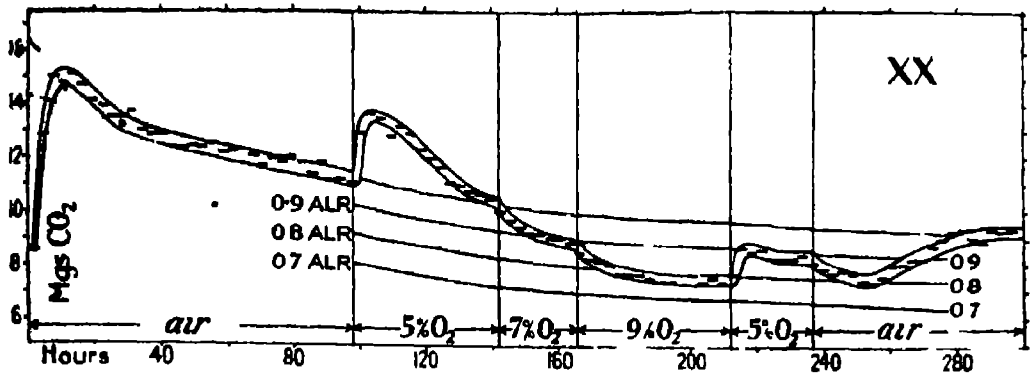


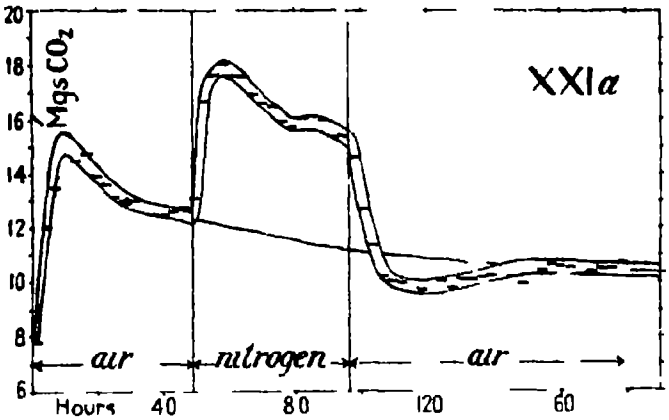


up above it giving the usual initial temperature effect These two records are the type for early apples of Class A

Record VII (see p 480) —This record was continued for 320 hours and even then showed but slight fall of the air line It is typical of early Class C apples showing a rising air line in the early part The exposure to nitrogen gives the typical Class C form but this does not recur until apple XXII In the middle of the nitrogen effect the nitrogen respiration values NR lie on the air line The track by which CO returns in time to the ALR after nitrogen shows a striking characteristically transitional form

Record VIII (see p 481) —This record was complicated by the active development of a patch of mycelium during the observation It provides the only case of this observed A full account of it is given in Section IV of the present paper To the mycelium is due the rapid rise of respiration after hour 60 Nitrogen kills the mycelium and the end of the record is believed to be pure apple respiration This apple is assigned to Class C The continuous line for CO_2 production between hours 215 and 237 is due to stoppage of clock work so that seven 3 hour readings were merged into one of 21 hours The exact course of the record therefore becomes uncertain at this point, and the

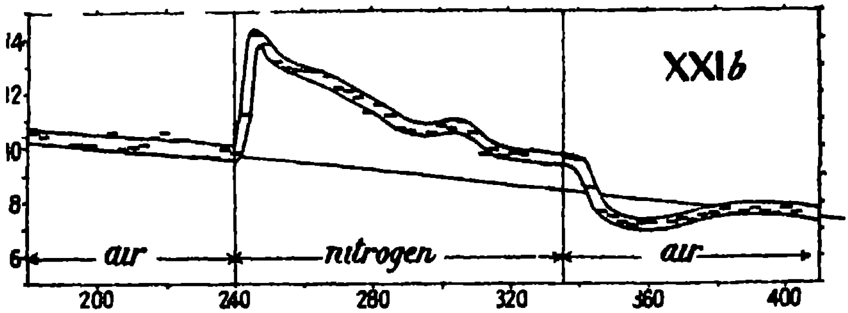


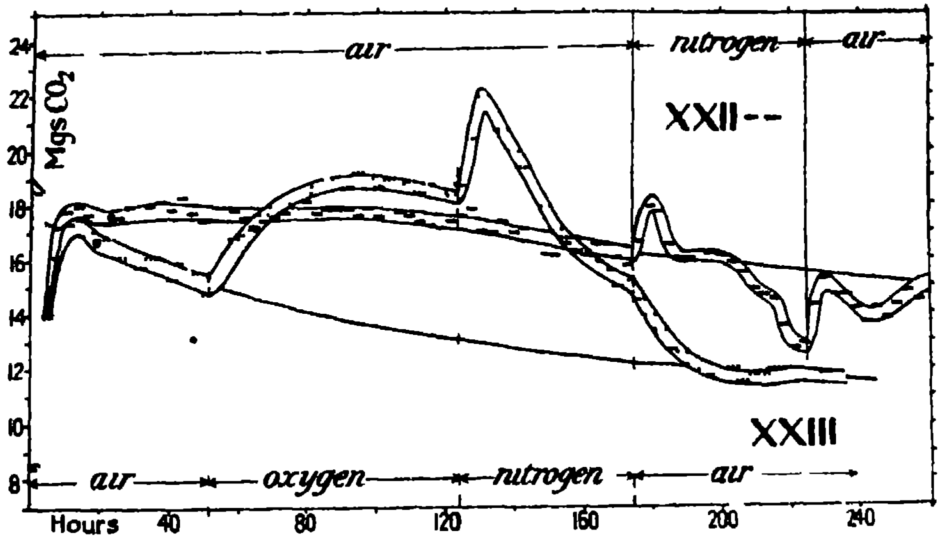


drift given to the contour lines here is based on the form observed in VII after nitrogen.

Records IX and X (see p. 481.)—These are two early apples of Class A. Apple X was kept in air throughout the record whilst IX was given 5 per cent. O_2 from hour 23 to hour 68. The air line of X closely resembles that of V and VI, having the composite form which after an initial level track curves down from hour 40 to hour 70 and then starts another level track before curving down again. Record X here serves as a 'control' for the behaviour of IX in 5 per cent. O_2. We see the depression of respiration by this low oxygen down to very much below the air value of X. On return to air IX rises in a slow smooth curve for about 45 hours and then regains the value of the control and proceeds afterwards along an identical track. We conclude that X represents also the course of the air line of IX.

Record XI (see p. 482.) This apple was treated with 5 per cent. O_2 after a rather short preliminary period in air and its respiration is depressed in the same way as in record IX. After 44 hours in 5 per cent. O_2 the apple was

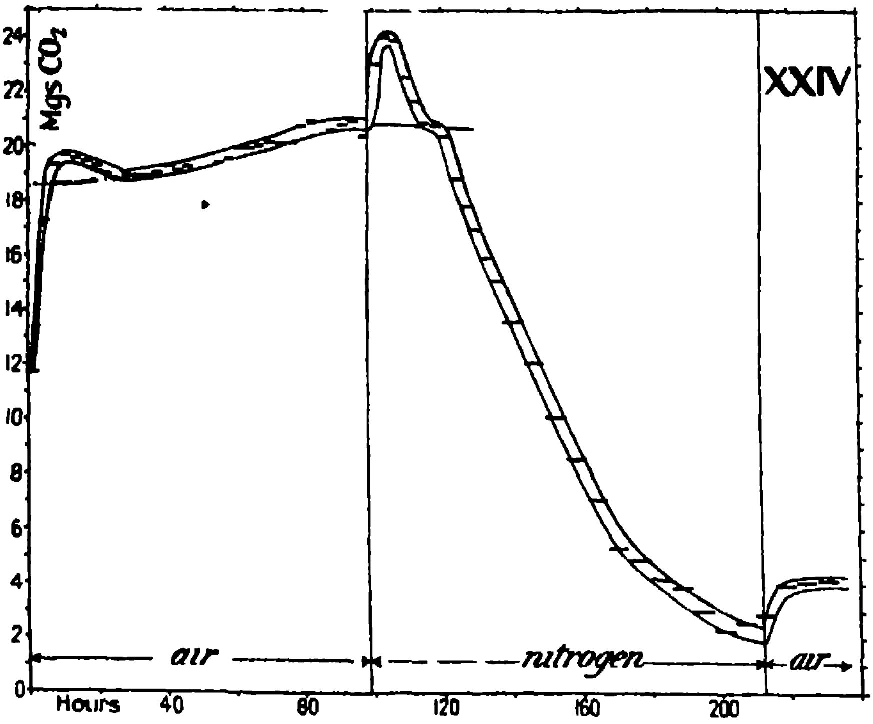


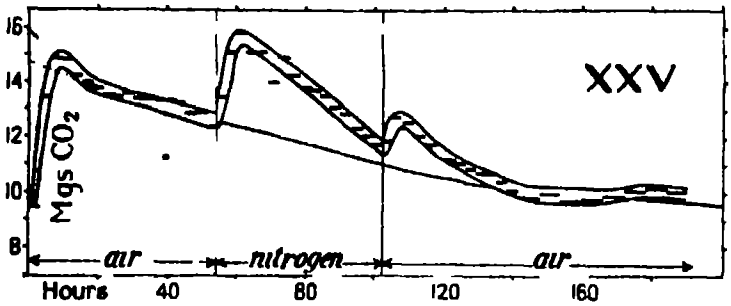


subjected to 39 hours in nitrogen without any interval in air This is the only case of such treatment and the interpretation of the record presents several uncertainties NR is clearly above the air line when properly adjusted to nitrogen and on returning to air the record dips far below the air line and then climbs up to it again These features are different from those of apple VII in nitrogen and mark this apple as belonging to Class A The form of drift of the air line is not well established as it requires an interpolation lasting about 130 hours The clockwork stopped between hour 99 and hour 114 and a single reading 15 hours long was alone available No other early apple of Class A was subjected to nitrogen so we have nothing to compare this record with

Record XII (see p 481)—This short record was in air throughout its course and gives an air line of the type of V-VI-IX-X It was carried out simultaneously with XI but we have provisionally adopted a later type of air-line for XI

Records XIII and XIV (see p 482)—These two Class B apples were





recorded simultaneously and subjected for periods to 3 per cent and 5 per cent O_2 In the preliminary period in air XIV lies appreciably above XIII, and its initial temperature effect is shown as lasting to a later hour Both low concentrations of oxygen depress the respiration but 3 per cent gives less low values of CO_2 production than 5 per cent relative to the air line The ultimate depression in 3 per cent is preceded by a preliminary rise of the CO_2 record above the air line an effect which is lacking in 5 per cent These features will be interpreted in detail in the fourth paper of this series The recovery of XIV after 3 per cent follows a tortuous course but ultimately it gives a location for the air line, drawn as a continuous line To XIII is assigned an air line, drawn as a broken line which is lower throughout and runs parallel to

XIV This location is supported by the values in air at hour 120 but the apple was not brought back to air at the end of its record

Records XV and XVI (see p 483) These two Class A apples were recorded simultaneously The first 30 hours of the records were spoilt by an experimental failure but the observed values from hour 30 to hour 48 have been extrapolated back to zero hour to locate the initial respiration values Apple XV was kept on in air till hour 140 so that its air line is extremely well established After this it shows a typical depression of respiration by 5 per cent O_2 but it was not returned to air Apple XVI was early given 3 per cent O_2 and shows exactly the same type of effect as records XIII and XIV After 3 per cent it recovers in air to give a track that is a perfect continuation of XV in air It is therefore concluded that XV and XVI have identical air line tracks after hour 40 before which XVI is slightly below XV At this stage of senescent development the air line approaches the type of a rectilinear decline throughout thus differing from the early type of V and VI

Record XVII (see p 483) This apple assigned to Class A was examined in 5 7 and 9 per cent O_2 without return to air The air line drawn for it in the chart is derived from the common line of the two immediately preceding apples XV and XVI To facilitate evaluation of the grade of depression below the air line which these various low concentrations of oxygen produce two construction lines have been added giving values for 0 8 ALR and 0 7 ALR respectively The effect of 5 per cent O_2 on XVII differs from that of the early 5 per cent effects with IX and XI and resembles rather that of early 3 per cent This is an outcome of advancing senescent drift At hour 12 the thermostat failed and the drop of temperature is shown reaching a minimum at hour 20 after which it was readjusted and recovery takes place The distorted readings in the chart are only connected by a mean line instead of by double contour lines The early piece of record shown in sets of three dots up to hour 45 is that of XVIII which was carried out simultaneously in air This is left in this chart to demonstrate the identity of the effect of temperature distortion There is a slighter temperature distortion between hours 108 and 120 when the temperature fell about 1° C throwing the observed values below the contour lines The dots in series above these displaced values represent temperature corrections to 22° C by use of $Q_{10} = 2\ 5$

Record XVIII (see p 484) This apple is assigned to Class B on consideration of its initial value of respiration and the form of drift of its air line which differs from adjacent Class A apples The apple was kept in air for 220 hours so its air line is perfectly established It was carried out simultaneously with

XVII and shows the same temperature distortions at hour 12 to hour 30 and hour 108 to hour 120 as we have just described

Record XIX (see p 484) In this record the effect of nitrogen was tried on an apple of Class A and we have a striking record showing how high NR rises above respiration in air On return to air the CO_2 drops below the air line before carrying out the characteristic recovery In this record once more the heating failed at about hour 138 The later low readings up to hour 150 have been omitted from the record as at this hour the experiment was discontinued and the air line lost The air line here has no direct support in its later region but with apples in this advanced stage of metabolic drift the form is well established by the next two records The initial temperature effect is exceptionally marked here

Record XX (see p 485) This also is a late apple of Class A and was used like XVII for investigation of 5 7 and 9 per cent O_2 Its air line is well established by a preliminary 100 hours in air and a final return to air for 60 hours at the end It shows the typical smooth falling curve getting less and less steep Construction lines for 0 9 0 8 and 0 7 of the air line values are added to facilitate estimation of the depression produced by low percentages of oxygen

Record XXI a (see p 485) This is the type apple of the late stages of Class A Its air line is very well established with long periods in air Nitrogen gives the typical effect with NR high above ALR and afterwards in air the CO_2 record dips below the air line before recovering its position on it

Record XXI b (see p 486) This record is a direct continuation of XXI *a* on the same apple but is separated from it because it contains a second exposure to nitrogen given after complete recovery from the first The two records together extend over 400 hours and show how complete is the recovery from even 96 hours in nitrogen The air line of XXI *a* is a falling curve slowly flattening out while in XXI *b* this has passed over into a practically rectilinear slope of constant decline

Record XXII (see p 486) With this record we encounter again an apple of Class C, which we have not met since VII and the air line drift rises slightly for a long time, so that after 100 hours it is still above the initial value Even at the end about hour 260 the fall is very slight The air line is well established by 170 hours in air at the beginning When nitrogen is given the CO_2 record displays a form of exactly the type of VII but not met since then with any of the nitrogen treatments The recovery from nitrogen in air is also exactly like that of VII

Record XXIII (see p. 486).—This record was carried through simultaneously with XXII and appears in the same chart. The divergence of its air-line from that of XXII is very striking, seeing that they are so close initially. This apple exhibits the falling curve type of air-line, and is assigned to Class B. This is the only case among the set of apples in which 100 per cent. O_2 was given, and it exhibits the striking increase of CO_2-production, which rises gradually till it amounts to 1·4 times the air-line value. At hour 125 nitrogen was given directly after oxygen, leading to a further increase of CO_2-production, high above the air-line, as in the A–B type. At hour 174 the apple is returned to air and the CO_2 record falls to the air-line, dipping just a little below it about hour 200, before the subsequent rise of values about hour 230.

Record XXIV (see p. 487).—Here we find the air-line rising during the first 100 hours, marking this out as a Class C apple. The record in nitrogen starts with the form typical of Class C, but the decline sets in quickly and steeply. Nitrogen was continued for 114 hours, and the continued fall and lack of adequate recovery in air demonstrates that a toxic effect has been brought about by nitrogen in this case.

Record XXV (see p. 487).—This record was carried through simultaneously with XXIV, but the form of the air-line is of the A–B type, like that of XXIII and unlike XXII and XXIV. The form of the record in nitrogen is also clearly of the A–B type, though the values fall off very fast. After nitrogen the record in air shows an initial rise of CO_2-production which does not occur in earlier apples of Classes A and B. This we attribute to a specific after-effect of CO_2-production. When this is over, in about 45 hours, the air-line is again reached.

Analytic Studies in Plant Respiration. III.—*Formulation of a Catalytic System for the Respiration of Apples and its Relation to Oxygen.*

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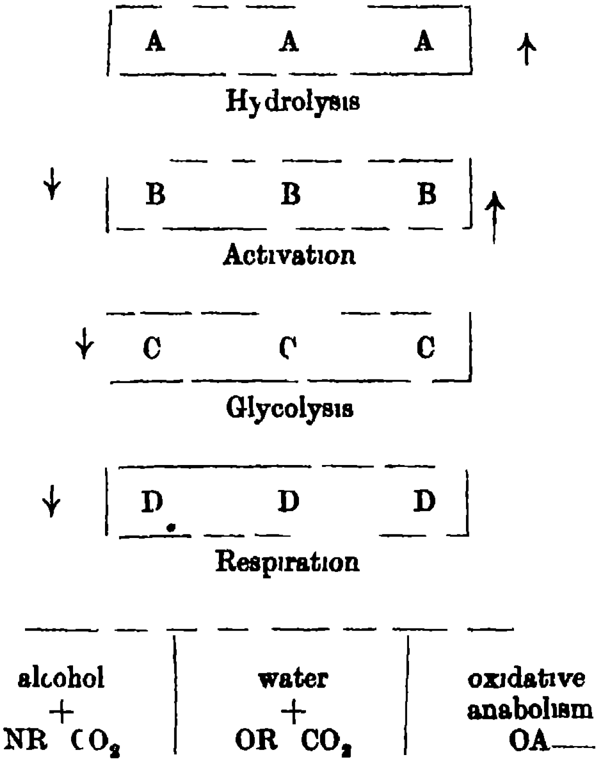
This paper is an extension of the analytic study started in the second paper of this series, which dealt with the respiration of apples in nitrogen. In Part I of that paper the phenomena of CO_2-production in nitrogen were described empirically, as they presented themselves in our records. Part II was devoted to examination of numerical relations, such as ratios and differences, that could be established between CO_2-production in air and CO_2-production in nitrogen. This present paper is, in effect, Part III of the analysis, but it starts out on new lines though use is made of various significant ratios established in Part II.

We here attempt a more realistic analysis of the phenomena than was possible when attention was concentrated merely upon CO_2-production. This advance in realism is based upon bringing into our survey the whole drift of the metabolites involved in respiration and picturing this drift as a system of catalysed reactions.

SECTION I —THE CATALYTIC SCHEMA PROPOSED FOR THE RESPIRATION OF APPLES

This system takes the form of a chain of reactions so that the products formed by one link reaction become the reactants of the next link At its free end the chain of reactions is branched and we find alternative fates for reactants controlled by the oxygen supply

We have simplified the system as much as possible but must take account of at least half a dozen catalysed reactions These will be formulated only for broad schematic treatment as our correlation of them is merely preliminary to fuller investigation It will save premature commitments to specific molecular reactants if we represent the substances and stages involved by a formal sequence of letters Our suggested schema is set out below



Schema of Reactions comprising the Respiration Sequence

The schema starts with a first group of reactants entitled A which includes all the substances in the apple which may function as reserves of carbohydrate and give rise by hydrolysis to free normal hexoses These normal hexoses constitute our group B We have assumed in our scheme that these normal hexoses are not respired directly but that a further carbohydrate stage intervenes, which we may call activation leading to the formation of hexoses of the group of heterohexoses with the less stable type of internal ring structure This group C is regarded as the direct substrate of the next reaction entitled Glycolysis

Here we come to the well known activity of the zymase complex leading typically to the formation of alcohol and CO_2 It is known that in the absence of oxygen apples do actually produce these products abundantly The study of this process in yeast shows that it is an elaborate complex with many intermediate products with two or three carbon atoms of the type of methyl glyoxal lactic acid pyruvic acid and acetaldehyde For the purpose of our simplification all these intermediate products of glycolysis are grouped together as D though ultimately it may be possible to differentiate them as D_1 D_2 D_3 etc Glycolysis here signifies the stage of conversion of reactant C to product D The products D are assumed to be the reactants for the last stage and so have alternative fates bound up with the presence or absence of oxygen This last stage we may speak of as Respiration in a narrow special sense In nitrogen group D proceeds quantitatively to the two final products CO_2 + alcohol in the usual ratio and these escape from the system as waste end products The CO_2 diffuses out and its production rate can be measured it will be held that for one atom of carbon thus detected there are two atoms of carbon excreted into the tissues as alcohol

We propose in this quantitative consideration of the workings of the system to adopt the carbon atom as unit and so evade specific molecules When previously we considered respiration of apples in nitrogen merely as a phenomenon of CO_2 production we termed this production nitrogen respiration NR and we shall maintain this nomenclature noting that when for instance it is stated NR = 1·5 the constant implication will be that this is a measure only of that part of the carbon loss appearing as CO_2 Taking into account the simultaneous production of twice as many carbon atoms in the form of alcohol it is clear that glycolysis = 4·5 is the exact equivalent of NR = 1·5

In air and other concentrations of oxygen the respiration system behaves otherwise and one of the main objects of our analysis on the present lines is to attempt to suggest a more precise formulation of the effect of oxygen than has hitherto been put forward The problems involved will be developed more fully later, after this preliminary sketch of the system is concluded.

It is known that oxygen has no direct effect on zymase activity in yeast, and we shall assume that in apples glycolysis is equally effective in any gas mixture in converting C to products of the group D. In air, however, the only detectable final products escaping from the system are $CO_2 + H_2O$. Now in apples we find that the CO_2-production by this process, which we label as OR (oxygen respiration) is less than the CO_2-production by NR. Clearly then the total carbon loss is three or more times as large in nitrogen as that in air. What then happens to this deficit of carbon in air? No final carbon derivative of D accumulates in the tissues during OR, so the logical conclusion seems to be that in air part of the group D is somehow worked back into the system continuously by oxygen. There is therefore a call for a third reactive mechanism, dealing with D, which we shall speak of as oxidative anabolism, OA. It is round this conception that problems are densest.

This anabolic building-back is specific to the presence of oxygen, but of course short-range up-grade reactions occur in those link reactions which are held to be directly reversible. Substances of group B can pass to A by condensation, and C can pass to B by reversion. In contrast with these early stages we shall assume that C → D is not reversible.

Other reactions than those set out are possible, such as the direct origin of C from A since many reserve carbohydrates contain heterohexoses in their structure. In yeast it seems possible that alcohol may undergo oxidative anabolism to carbohydrate, but the evidence available for apples indicates that alcohol once formed in the tissues remains unalterable.

SECTION II.—A GENERAL SURVEY OF THE WORKING OF THE PROPOSED RESPIRATION SCHEMA.

This system of linked reactions brings together into one sequence processes generally regarded as belonging to separate chapters of physiology, and combines matters of carbohydrate equilibrium in tissues, stages A–B–C, with processes more specifically connected with respiration D–NR–OR. The whole system therefore presents a number of rather complex relationships and it may be well to give a general survey of its supposed workings before trying to relate it quantitatively to the actual data of our respiration records. This latter aspect will be the subject of the following sections.

§ 1. *The General Drift of Respiratory Activity in Starvation.*

Taken as a whole the schema is intended to represent the fundamental continuous catabolic drift of carbon compounds from the highest molecular structure and energy-content towards lower states of these attributes, beginning with production from reserves at the top and ending, at the bottom, with excretion of respiratory waste products.

All along the chain the drift is determined by balance of production and consumption. The controlling factors are the activity of the catalytic components of the system and the amounts of the substrates available. When such a system is studied in a living tissue isolated from the plant and no longer able to increase the amount of A, then we are in the presence of what we may call, broadly, starvation phenomena, but the course of these, when followed in time by measurement of the respiratory products, may appear very different in different tissues. One fundamental variable is the amount of A in relation to the activity of conversion of A to B, and another the *absolute* activity of the final respiratory catalytic system. The apple is distinguished by having a very inactive respiratory system in relation to the amount of A and B, so that the isolated tissue may maintain the system in normal working for many months, or even a year, on its original stock of A.

When the apple is freshly gathered in autumn its respiration may be low and then proceed to rise, in spite of isolation, a phenomenon which we have discussed in the first paper of this series, and attributed to a "decrease of organisation-resistance" which is effectively an increase of "hydrolysis-facility" leading to more rapid production of C from A and B. This brings about a rise of respiration and greater excretion of final products out of the system. In the later stages of isolation in storage, to which belong the apples now to be considered, this rise of respiration is over and the whole system exhibits steadily declining magnitudes of catabolic drift and decreasing respiration. This decline is, at 22° C., at first relatively rapid, but it gradually slackens off, appearing to reach in time a slow rectilinear fall. Of the form of such a decline, the air-line of apple XXI, *a*, *b*, is taken as a typical example. The average intensity of respiration in this starving case is about 10 mg. CO_2 per 100 grms. apple per 3 hours. Calculating this as hexose oxidised per day we find that the loss is only 0·057 grms. Assuming that the apples still contain 10 grms. hexose per 100 grms. tissue, the daily loss is no more than 0·5 per cent. of the stock of B. In addition there is the production of B from A to compensate this consumption, so that the drift of starvation grade from day to day is very slight and we almost approach a state of dynamic equilibrium. Nevertheless we must recognise that the system is always progressively starving, and the excretion of carbon at the distal end always somewhat greater than the initial production value, which lags behind it. The steady states of this nature, in which the rate of respiration is determined by production at the top we propose to distinguish as "adjusted states" of the system.

Since we have already established that the loss of carbon in nitrogen may start as high as 4·5 times the loss of carbon in air, we see that on change from one gas to the other we do not merely alter the nature of the biochemical mechanism but also we suddenly change the carbon drift quantitatively and alter what we may call the starvation-grade. We want, therefore, to find out how the chain and its separate links react to this sudden alteration of consumption at the free end. Realisation of this quantitative difference between NR and OR has helped to drive our thoughts back to the production of carbohydrate substrate for respiration, and led to our linking up carbohydrate metabolism and respiration into one production-consumption system.

The sudden change of carbon loss produced by alteration of oxygen supply we view as a sudden disturbance of the carbon traffic system, displacing it from the previous adjusted state. If the new set of conditions is maintained long enough we may expect the system to settle down to a new adjusted state. The relations of the various adjusted states in different conditions of oxygen supply should illuminate the control mechanism of the transport system, as should also the character of the transitional unadjusted states through which the system passes in moving from one adjusted state to another. All these changes are slow in apples because the activity of the catalyst systems is, absolutely, slight. The result of this is that long tedious experimentation is involved in ascertaining the relation of final adjusted states, but there is the compensating advantage that the transitions are of a comfortable slowness so that they can be followed in considerable detail. Tissues provided with a more active catalytic system might pass from one adjusted state to another too quickly for experimental determination of the course of the transition. We shall learn much from the forms of transitions.

§ 2. *The Reaction of the Respiratory System to Alterations of Oxygen Supply.*

Having sketched the carbohydrate starvation aspect of the system we may proceed to enquire into the more strictly respiratory processes and the influence of oxygen. It will be proved in the next paper that the intake of O_2 and the output of CO_2 are increased by rising external oxygen concentration. Details are reserved until that exposition, but we need here to realise that if the respiration in air is taken as unity, then that process steadily accelerates with rising oxygen up to a value of 1·4 in pure oxygen and steadily falls off with decrease of oxygen till it reaches a value of 0·7 or less, in 5 per cent. O_2. As we have found in our present study of nitrogen that CO_2 in nitrogen is greater than CO_2 in air, we perceive that there must be a minimum of CO_2-production, though not of oxygen intake, in some one low concentration of O_2. In apples this occurs in the region of 5 per cent. O_2.

In face of these facts, our first tendency was to regard the increase of respiration with increased supply of O_2 as due to increase of the respiratory oxidation by the greater concentration of one of the substrates of respiration, namely, oxygen. More analytical consideration of the data available tends to lead us away from regarding this as the fundamental interpretation. Our present object is, then, to enquire exactly what changes oxygen does produce in the system.

For analytic treatment of the system it seems to us that the long chain of reactions must be divided into three separate short chains, though each will be linked to the next by the fact that its own end product is the primary reactant of the next stage.

The first stage is the carbohydrate one, A–B–C, concerned in the production of C. The second is the glycolytic system concerned in the production from C of the intermediate products D, and the third is the oxidative system concerned in production of final products OR and OA from D. Unlike the others this last stage can be thrown entirely out of action by nitrogen so that zero activity of it with substitution of NR comes into the possibilities. This being so, we shall provide ourselves with a more general formulation by defining our three stages as (1) glycolysis, (2) stages antecedent to glycolysis, and (3) stages subsequent to glycolysis. This analysis seems to us profitable, because our data lead us to the conclusion that the relation to oxygen is different and characteristic for each of the three stages.

The Relation of Glycolysis to Oxygen.—It is well known that oxygen has no direct effect upon the zymatic glycolysis of sugar in yeast, so we should approach this matter with the expectation that the same would be true of glycolysis in the apple. By glycolysis, we understand here the conversion of the substrate C to the products D so that the term has only the narrowest denotation. The first problem is the experimental determination of the magnitude of glycolysis in the different circumstances. In nitrogen the apple produces alcohol as well as CO_2, and if we assume that the proportions of these are the usual ones then it is clear that the measure of glycolysis in nitrogen is, in terms of carbon units, threefold the observed CO_2 in nitrogen. So we can always write for nitrogen, Gl = 3 NR. For estimation of glycolysis in air or oxygen mixtures we have no such direct measure. As OR in air is found to be much less than NR we might draw the superficial conclusion that oxygen had reduced glycolysis considerably.

On the Pfefferian view of aerobic respiration it would be assumed that in air the whole of the carbon of glycolysis appears as CO_2, while in nitrogen only one-third of the carbon is constituted by CO_2; thus the conclusion that oxygen reduced glycolysis would be inevitable. However, we have satisfied ourselves that oxygen does not have this effect and we consider that we can employ an experimental procedure which will enable us to arrive at the actual magnitude of glycolysis that is being carried on by an apple in the adjusted state in any oxygen concentration.

The procedure is this: the oxygen current is suddenly replaced by a current of nitrogen and the CO_2 output measured hour after hour. It is found that the CO_2 rapidly rises to a high level and then steadily declines for a long time. This declining series gives a point to point measure of NR and of glycolysis. If now the series of values be extrapolated backwards to the zero hour of entrance into nitrogen, we get a measure of what the glycolysis would have been at zero hour could the transition have been made instantaneously, before starvation had progressed further. This initial value is the value of glycolysis that was going on actually in the adjusted oxygen state previously. We get full confidence in this evaluation of glycolysis when we find that for a given physiological state this initial NR value has, every time, the same ratio to the last OR value at the moment of change into nitrogen. Subsequent behaviour at the transition, on bringing the apple back to air from nitrogen, confirms our estimation of this magnitude of glycolysis in a way that will be brought out clearly in a later section on transitional relations, p. 504. Each "initial value" of NR in nitrogen that has been under discussion in Part II of the previous paper supplies us then with a measure of the glycolysis in air or the other oxygen mixture that preceded the nitrogen. It results that we have at our disposal in that paper, material for evaluations of glycolysis in oxygen mixtures in quite a number of the records, based upon the careful examination of their transitions into and out of nitrogen. These glycolysis records will be set out graphically later in this paper.

This we consider an important advance, as in measuring glycolysis we are measuring the common antecedent stage to both types of respiration and so get the common measure of both NR and OR, or of a mixture of OR + NR. These evaluations of glycolysis establish that glycolysis is really maintained at a higher rate in air than it can be maintained in nitrogen; and further, that glycolysis is 1·4 times as high in pure oxygen as in air and is depressed to 0·7 of the air value in 5 per cent. O_2. The facts show that glycolysis moves up and down with oxygen concentration just as OR does. The magnitude of glycolysis relatively to OR is high, so that for some classes of apples Gl = 4·5 OR while for others Gl = 4·0 OR.

Were it not for our knowledge that oxygen does not increase the rate of glycolysis in yeast we might have been inclined to suggest that in some way or other oxygen increases the efficiency of conversion of C to D. As this increase of glycolysis by oxygen is not merely a transitional effect, but leads to a permanent increase of adjusted rate it is clear that the production of C as the substrate for glycolysis must be permanently increased by oxygen. We have therefore before us the possiblity that oxygen has no direct effect on glycolysis at all and that the rise of this functional activity in oxygen is merely the necessary outcome of the increased production of C. To this we shall return shortly.

The Stages subsequent to Glycolysis and their Relation to Oxygen.—We have now to concern ourselves with the fate of the D group of substances which are the products of glycolysis and the reactants of the final stages of respiration. Our schema provides for their conversion either to NR, OR or OA. If we start our survey of oxygen effects with an apple in nitrogen we can regard the situation as simple: the whole of D that is being produced is converted to alcohol and CO_2 in the usual ratio, so that the consumption of D equals 3 NR measured in carbon units. What then will happen when traces of oxygen are admitted by some low oxygen concentration outside the apple? We have already satisfied ourselves that this oxygen will not lower the rate of production of D, but nevertheless we find as a fact that the production of CO_2 falls off markedly, carrying with it, we assume, a similar drop in the production of alcohol. Now the substances of the D group are not autoxidisable so we naturally assume that the specific catalysts of oxidation are now able to start activity and compete with NR for D, oxidising these substances to the extent that the oxygen supply can permit. We picture this oxidation as a reaction of high oxygen affinity and irreversible so that the consumption of the available oxygen is complete. As a result there is produced a certain amount of OR and OA. The totality of CO_2 escaping will now be partly derived from NR and partly from OR, so that we have use for the expression TR = OR + NR (see 'A. S. R.,' II, p. 460).

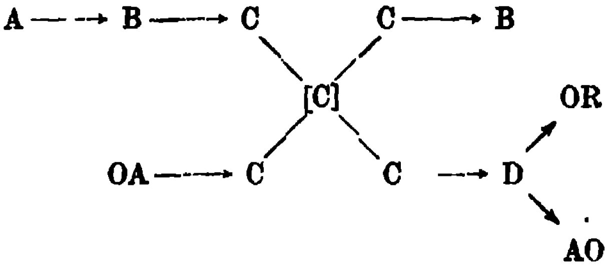
With the right external low concentration of oxygen it can be arranged that, say, half D is converted to NR + alcohol and half to OA + OR. With a still higher external oxygen concentration a point can be reached such that just enough oxygen enters the cells to convert the whole production of D to OA + OR and there is now no longer any NR production. This marks a definite physiological state, and one experimental task in the next paper will be to locate the external concentration of oxygen that, for a given tissue, coincides with this "extinction point" of NR. One physiological index of it, in a living apple, may be that at this point there is a minimum production of CO_2. Incidentally it has the significance that at this value there is presumably no longer any accumulation of alcohol in the tissues. There will be further consideration of extinction points in the next paper on oxygen. According to this view a higher concentration of oxygen, such as air provides, cannot carry out any more active oxidation of D, for by definition D is wholly oxidised to OA and OR by quite low supplies of oxygen, such as obtain at the extinction point, usually located in the region of 5 per cent. external oxygen. In accordance with this we put forward the view that in any moderate concentration of oxygen the whole of the CO_2 is to be labelled OR, without any NR component. We have next to consider the magnitude of the OA production that is associated with this OR.

The Magnitude of Oxidative Anabolism in Respiration.—We propose to treat OA as a substance, but it must be made clear that as far as evidence goes it is only a magnitude. In the section on glycolysis it was shown that by the application of a particular canon we claim to be able to measure glycolysis in each adjusted state of respiration in oxygen mixtures. Obviously we can measure OR in the same state, and OA is only the amount of carbon by which produced CO_2 falls short of carbon glycolysis. The amount is large, and as far as we have yet experimented we find that, for a given physiological state and class of apple, OA bears a constant ratio to OR. The extremes of this ratio observed for different classes of apples range from OA = 3·5 OR to OA = 3·0 OR. With a given state of the apple this ratio seems to be independent of the magnitude of glycolysis and of the concentration of oxygen. So we adopt the view, provisionally, that the two substances are colligate parts of the products of one catalytic system and not the result of two independent catalysts working on D as substrate.

The next question to be faced is what becomes of OA thus continually being produced in oxygen mixtures, for there is no evidence of any down-grade product accumulating in the tissues. The symbol we have given to it is based on the assumption that it is anabolised back into the carbohydrate region of the system antecedent to glycolysis, so that possibly the same carbon atom may circulate round through the glycolytic machinery several times before it chances to be thrown out finally as CO_2. It might be that OA passed directly to the highest carbohydrates of group A, or to normal hexoses of group B, or, on the other hand, it might arrive at the bottom of the series in group C. As the amounts of A and B are very large, it would not make a significant difference to the working of the system should the return of OA to them be cut off for a period of hours. But should its destination be C, which can only be present in small amount, then the arrival or non-arrival of the OA contribution should make its mark on the concentration of C and therefore on the rate of glycolysis, which is a measurable function. It must be emphasized here that though our hypothetical substance OA is regarded as an outcome of the oxidation process OR, there is no reason to regard it as being necessarily an *oxidised* derivative of D. It might indeed be a reduced derivative, colligate with oxidised products $CO_2 + H_2O$. If that were so the catalytic process would be an oxido-reductive process and the reduced component be carried back to carbohydrate. A study of the actual oxygen consumption in all critical stages of apple respiration, which has recently been started, may help to throw some light on this uncertainty.

Finally, in this connection, we have to refer to the existence in apples of a fair amount of a metabolite which is not a carbohydrate, namely, malic acid. If malic acid is to be identified with our OA it would be necessary to hold that this substance is always being rapidly produced and consumed, since it does not accumulate in starving apples, but diminishes slowly. The amount present at any one time would be the difference between the rate of production from D and the rate of consumption by the combined possible fates of further oxidation to OR and anabolism to A, B or C of the carbohydrate group. The amount of malic acid in the tissues can be directly determined, but its variations under change of oxygen concentration have not yet been established. There is a possibility of getting a further contribution to exact knowledge in this direction.

The Stages Antecedent to Glycolysis and their Relation to Oxygen.—The essential point for examination here is: does oxygen increase the production of C and, if so, through what mechanism is this brought about? The tendency of all the previous sections has been to make the concentration of C the real nodal point in the functioning of the catalytic system which we have schematised. An analysis of the rates of production and consumption of C is complicated by the possibility that no less than four reactions may be involved here. These are set out in schematic form below



Two of them are founded on the expectation that the B — C relation must be a reversible one. A third is the irreversible glycolytic conversion of C to D, while the production of C from OA provides a probable fourth reaction. In nitrogen this set becomes simplified to three, for D goes wholly to NR, and no OA is produced. This power of cutting out OA by nitrogen is one of the experimental fields to which we can look for evidence as to whether OA should be held to pass to C. Any excessive production of C above a balanced state would lead to increased transport in the direction $C \rightarrow B$, so that OA would find its way to B ultimately, and perhaps even to A. This distinction between the effects of OA and B as sources of C has been given some consideration, but as the specific velocities of the reactions concerned are unknown we must postpone the enquiry till the initial stages of transitions have been worked out for successive short periods, instead of the 3-hour periods employed in the present investigation.

By subjecting an apple to pure oxygen instead of air we have established, that glycolysis together with OR and OA are collectively increased 1·4 times, all keeping their relative proportions of $4 \cdot 5 = 1 + 3 \cdot 5$. To maintain this increased adjusted rate not only must C have a higher concentration but the production of C from $A \longrightarrow B \rightleftarrows C$ must be increased in the ratio of 1·4. Whether OA passes back directly to B or at first appears as C does not affect the *adjusted* rates we are concerned with, though it might affect the precise form of the transition from one adjusted state to the other. We conclude from the above observations that the different oxygen concentrations in the cell bring about different rates of activity of the $A \leftarrow B \rightleftarrows C$ reversible mechanism. Theoretically the effect might be attributed to depression of $C \longrightarrow B$ or acceleration of $B \longrightarrow C$.

This effect may not involve the continuous chemical consumption of oxygen and it might be attributed to activation of a catalyst system or to lowering of organisation-resistance. The change to the new rate of activity after alteration of external oxygen is extremely slow, lasting some 45 hours, during which time the OR output is observed to creep up or down to the new level in the form of an equilibration curve, so that at first it approaches the new position fairly fast and then slower and slower (halving time about 7·5 hours). Such a slow rate of adjustment is characteristic of altered carbohydrate balance relations rather than of a direct chemical effect of altered rate of oxidation.

The implication, from all this, that the production of C from A and B is a function of the oxygen concentration, requires one marked qualification as regards very low oxygen supply. Without this qualification it might be falsely assumed that in complete absence of oxygen there would be no production of C from B, and therefore no glycolysis. These processes, however, proceed at a considerable rate in nitrogen; but this rate does not appear to be increased by very low supplies of oxygen, but remains about the same minimal rate till the oxygen supply determining the "extinction point" of NR is reached, say, 5 per cent. external O_2. It is conceivable that up to that point, till NR ceases to be able to exist, the affinity for oxygen is so great that practically zero partial pressure of oxygen is maintained in the cells, so that only from the extinction point onwards is there sufficient free oxygen to begin to cause that increase of C-production from B which we have just attributed to it. The facts on which these considerations are based will be found in the section of the next paper which deals with low oxygen concentrations. The interpretation of this absence of effect with low oxygen may, however, turn out to be metabolic.

We suggest that every increase of supply of *free* O_2 to the cell which raises the partial pressure of O_2 above zero increases the rate of action of the pre-glycolytic carbohydrate stage in the direction of increasing the production of the substrate C for glycolysis. An inevitable consequence of this is that glycolysis increases and more D is produced.

When we pass from higher oxygen to lower, then the glycolysis rate undergoes a corresponding diminution which is seen in progress, in its simplest form, in the passage from air to 5 per cent. O_2. When we pass from air to zero per cent. O_2 the same lowering of glycolysis takes place, but the evidence for it is obscured by the simultaneous big change in CO_2-production, due to the effect of lack of oxygen on the post-glycolytic oxidation stages.

In order to make a clear analysis of the complex changes that are at work during transitions into or out of nitrogen, a full Section will now be devoted to a formal analysis of the varieties of transitional phenomena that our records present

SECTION III —THE FORM OF TRANSITIONS WITH CHANGE OF OXYGEN SUPPLY

One of the outstanding characteristics of this investigation of respiration is the long duration of the individual records We obtain records of CO_2 production lasting many days and these show that after a change of oxygen condition the respiration presently settles down to give a steady drift of values which is sometimes level but usually presents a gently declining curve These states we speak of as adjusted states and in them all the stages of production and consumption throughout the schema are proceeding at a practically uniform rate When the oxygen supply is suddenly changed we get phases showing a marked and sometimes a violent change of CO_2 production which phases presently come to an end in new adjusted states These phases we speak of as transitional and the forms of the transitional records call for a special survey as it is from them that we have derived suggestions for many of the points elaborated in this exposition

There appeared on our first inspection to be two types of transition and it was a long time before we could provide an interpretation of their common features and their distinctive features We may label them as the *simple* type and the *complex* type The former manifested itself when a change was in progress from one intensity of OR to another intensity of OR while the latter characterised changes which involved NR such as change into nitrogen or out of nitrogen There are many cases of the complex type in the data of the present paper most of the simple transitions come up for treatment in the next paper but two occur among the present data These appear in fig 1 as the first transitions of the two records Apple XXIII goes through one on the change from air to pure oxygen and apple XI on the change from air to 5 per cent O_2 In XXIII there is an increase in OR from the air rate to the higher adjusted rate for oxygen which is 1 4 times the rate for air in the latter a decrease to adjusted rate for 5 per cent O_2 which lies at 0 73 the rate in air

In the case of XXIII there is some clue to when the rising transition is over because as soon as the adjusted rate is attained the record begins to decline The transition is thus seen to be very slow lasting at least 45 hours It is hard to believe that it would take 45 hours for a pure oxidation rate to adjust itself after an increase of oxygen concentration and this is one reason why we attribute this observed change to carbohydrate metabolism The form of the rising transition is exactly that of an approach to a reversible equilibrium, rising at first steeply and then slower and slower till the equilibrium state is

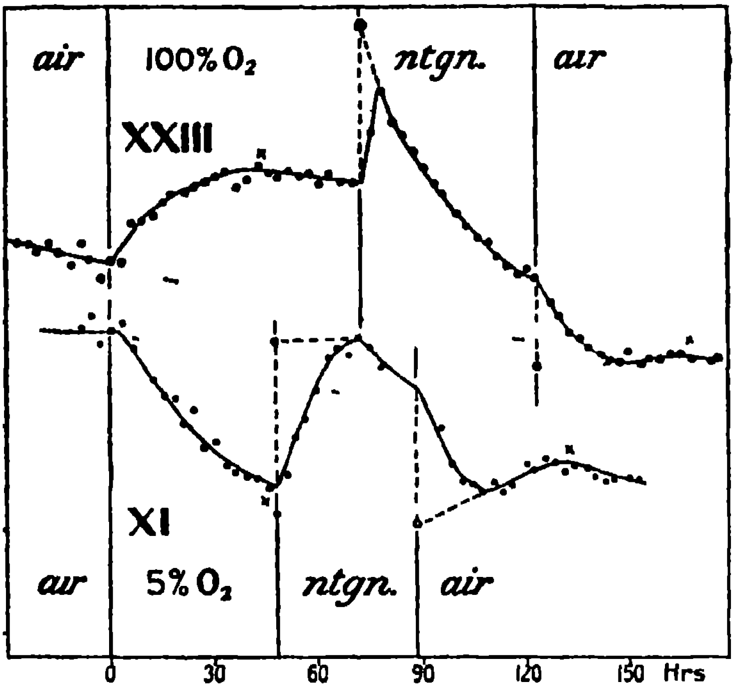


FIG 1 —Records to illustrate the forms of CO_2 production line at transitions The single 3 hour readings are represented by heavy dots and these are connected up by a smoothed median line to bring out the form The divisions on the ordinate axis are 4 mgs CO_2 apart the actual values are not given here but will be found in the full records in the Appendix to the preceding paper The course of ALR is represented by groups of three small dots The time period of 45 hours after the beginning of certain transitions is marked by a cross close to the record Each record presents three transitions the first on each does not involve NR and therefore exhibits the *simple* type The other two exhibit the *complex* type in which there is, in addition to the slow OR transition a comparatively sudden change of post glycolytic oxidation due to sudden appearance or suppression of NR with its higher production of CO_2 This sudden alteration to a new CO_2 level is indicated by the vertical broken line leading upwards or downwards to the circle which represents the initial value of the new state These initial values are masked by the physical lag in CO_2 escape so that the extremely high or low early values cannot be observed These are represented by the broken line after the initial point which presently joins on to the track of actually observed values Record XXIII is typical in the form of its transition into or out of nitrogen, resembling the cases in fig 2, but XI shows special features in its transition from 5 per cent O_2 to nitrogen which are discussed on p 517

reached. We picture the opposed processes to be increased production of C from A—B by oxygen activation, working against increased consumption of C by glycolysis, which rises with each rise of concentration until the two become adjusted to equality again.

In the case of apple XI, the lower record in fig. 1, the transition is downward from the air-line to the lower adjusted line for 5 per cent. The form of the transition is exactly the same as that for XXIII only the movement is all in the opposite direction. Though the end of the downward transition to a lower falling line is not so clearly located it is fairly well determined that this also lasts at least 45 hours. In confirmation of these forms of the simple type of transition we have observed the return from pure O_2 to air as proceeding just like the passage from air to 5 per cent. O_2, and the return from 5 per cent. O_2 to air like the change from air to 100 per cent. O_2.

When we observe transitions from air to nitrogen or the reverse we meet the complex form (see the first transitions of XXI, *a* and XXI, *b* in fig. 2). Though the change from air to nitrogen involves a lowering of the rate of production of C from A—B just as air to 5 per cent. O_2 does, yet the first effect on the CO_2 record is a rapid rise which comes to a end in about 9 hours and is then followed by a long continued fall in a curve of decreasing steepness. Every case of nitrogen given to an apple of Class A or B provides an example of this. When the nitrogen is discontinued and the apple returns to air the record instead of returning smoothly to the air-line, sweeps below it in 7–10 hours or so, and then slowly climbs back to the air-line, as the third transitions in fig. 1 and the second in fig. 2. We have now satisfied ourselves that these forms represent the additive product of no less than three separate transitions, each with a different timing, and it is to this that they owe their complex form. Let us enumerate these three changes.

It is clear that the change from nitrogen to air should produce the same transitional type of pre-glycolytic increase of production of C from A—B as we have just fully described for the simple transition from air to oxygen. To this must be added a second transition due to the sudden entry of oxygen which proceeds at once to divert all D from its previous fate as NR to its new fate as OA + OR. As in this class of apple, D, when undergoing NR gives 1/3 of its carbon as CO_2, and when diverted to OA + OR gives only 1/4·5 of its carbon as CO_2, there must be a sudden drop in CO_2-production within the tissues to 2/3 of its previous rate. This process if it stood alone would have as its transition an almost sudden drop of CO_2-production. The combined transition of these two would take the form of a sudden drop of CO_2, due to the post-glycolytic oxidation change, down to a value which was 2/3 the last NR value before the admission of oxygen, and from this low point there would develop the slow rise of CO$_2$ lasting 45 hours due to the simple transition of increasing C in the pre-glycolytic stage This combined form is drawn in figs. 1 and 2 as a broken line dropping vertically to a circle locating the new theoretical initial and thence rising slowly towards the new adjustment

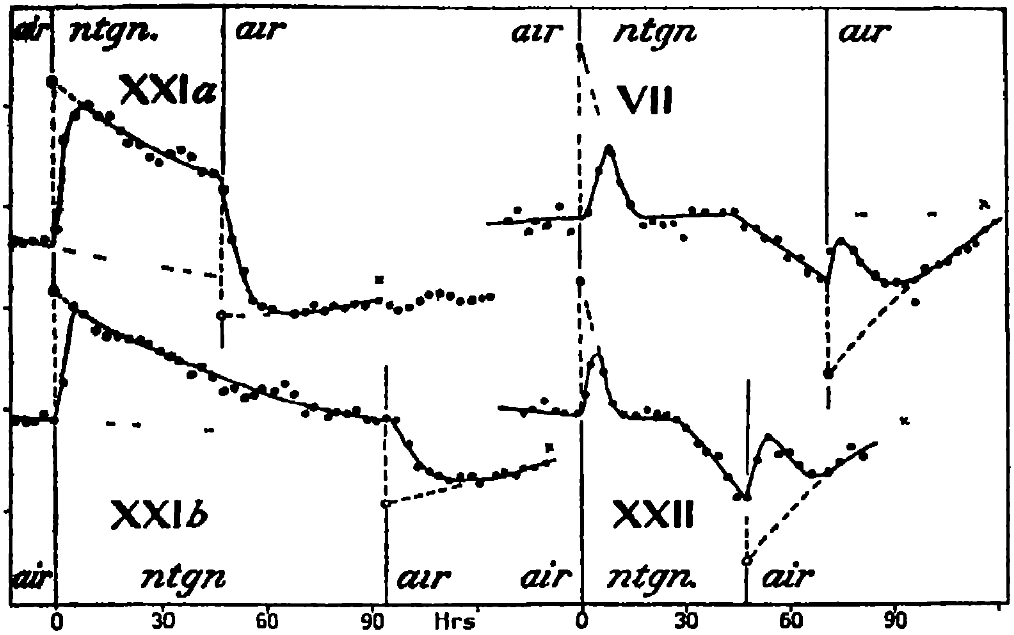


FIG 2 —General description as in fig 1 Records XXI, *a* and *b* exhibit typical complex transitions The transition air to nitrogen for VII and XXII is also of this type but the transition from nitrogen to air contains here, an ' after effect of NR " giving a small temporary extra production of CO$_2$ The last two cases being Class C apples, are described later (see p 517)

The third transitional effect which comes in to mask the combination of two just described is a purely physical affair, due to the long time it takes to equilibrate the CO$_2$ content of an apple with that of its environment by diffusion across the surface, which offers a considerable resistance If the internal production of CO$_2$ were cut off suddenly and completely, the store of CO$_2$ in the watery tissues would go on escaping giving a geometrically decreasing curve of CO$_2$-escape to the exterior, and it would be very many hours before this had all escaped to the air current During all this time there would be an appearance of decreasing CO$_2$ production by the apple The same form of CO$_2$-escape, though in a rising sequence, would accompany any instantaneous increase in actual metabolic production, making the rise appear slower than reality, and thus there is always an external distortion of the true form of the change of rate of internal CO$_2$-production. It follows that the sudden drop in the CO_2-production rate, due to the change from nitrogen to air, reveals itself only as a declining rate of escape of moderate duration, taking at least 10 hours and often more in an imporous apple till the new lower rate of actual production is attained.

Combining this third transition which is the physical CO_2-transition with the sudden oxygen transition lowering the metabolic CO_2-production from D and with, also, the glycolytic transition, *i.e.*, the slow rise of glycolysis due to increased production of C, we can build up exactly the observed form of transition in CO_2-escape from the apple which has been recorded many times at the change from nitrogen to air; see records XXI, *a*, XXI, *b*, XI and XXIII in figs. 1 and 2.

These triple complex transitions are not quite the end of the complication, for in several living tissues other than apples that we have studied, the most marked effect of air after nitrogen is a very large sudden production of CO_2, due to the oxidation of some accumulated product of NR metabolism. This we speak of as the "after-effect" of NR. In most metabolic states of apples there is no trace of such an after-effect, but apples of class C show a very small after-effect (see records VII and XXII in fig. 2), and so also does the very late apple XXV of Class B. In such cases there is a temporary rise of CO_2-production at the transition, maximating almost at once and subsiding in about 24 hours, just in time to reveal the last stage of the triple complex transition that we have described in detail. The end of these various transitions is that at last the CO_2-production works its way up to the adjusted air-line value ALR, and then drifts slowly down it in continuation of the original direction of the line before nitrogen was given. The nitrogen experience is at last, after a couple of days, a thing of the past which has left no permanent effect.

The contrary transition, from air to nitrogen, to which we now turn should clearly be built up as a complex whole from the three constituent transitions, all working in the opposite way. And this is what it proves to be. In the two figures the broken line course and the circle set out the proposed interpretation of these transitions. There is the quick oxidation transition, which at the cutting out of oxygen by nitrogen should increase the CO_2-production from D suddenly to 1·5 times its OR value. But this high initial value is not actually attained on account of the physical CO_2-transition, which makes a lag of about 10 hours before the rapidly rising CO_2-escape gets to its highest value. All this time the production of C from A—B is slowly declining towards the low rate characteristic of nitrogen. If there were certainty as to the direction and ultimate course of the "adjusted nitrogen line" towards which the transitional complex is working, then we could determine when the whole transition should be judged to be over. Certainly the falling NR series is still slackening off at hour 45, and even at hour 100 it is not certain we have a true adjusted rate. It may be that in time there is a toxic effect of accumulated alcohol and that no really adjusted rate can be maintained. Therefore at present, till longer records in nitrogen have been obtained we propose to speak of the nitrogen values after 70 to 100 hours or so as giving a "semi-adjusted" nitrogen line.

So far we have only dealt with the nitrogen transitions of apples of Classes A and B. It will, however, not have been forgotten that Class C presents us with nitrogen effects that at first seemed to be quite different. In reality these also can be satisfactorily interpreted on exactly the same analytic lines. Cases are presented in fig. 2, records VII and XXII, but we shall not bring them to account here as a full treatment of them will be found in connection with the study of glycolysis in apples of Class C at the end of the next section of this paper (see p. 517).

SECTION IV.—THE TRANSITIONAL CO_2-RATIOS OF THE INDIVIDUAL RECORDS AND THE CONSTRUCTION OF THEIR GLYCOLYSIS LINES.

Having provided an interpretation of all the features of the CO_2-records at transitions from one gas mixture to another, distinguishing the effects of oxygen on pre-glycolytic carbohydrate phases from the effects on post-glycolytic oxidation phases, we can now enter upon a further development. This takes up the problem of evaluating the rate of glycolysis in the different individual records, on the basis of their individual transitional CO_2-ratios.

The important new line that we claim to be able to add to these records is the *Glycolysis Line*, which sets out the rate of the glycolytic conversion of C to D throughout the whole course of the record, whatever may be the gas mixture surrounding the apple. The theoretical significance of glycolysis rate has already been elaborated. We regard it as a measure of the concentration of its substrate C, which is the outcome of the production activity of the carbohydrate metabolism chain A—B—C. The form of this line therefore reveals not only the general effect of starvation drift in lowering activity, but also the special effects of oxygen, air, and nitrogen upon the rate of glycolysis. By concentrating attention on glycolysis we get away from the distractions of the different CO_2-production ratios of NR and OR and contemplate the influence of the various oxygen mixtures upon metabolism apart from oxidation. From this point of view CO_2-production serves merely as an index of the rate of glycolysis, but the index value has to be weighted differently according to whether it is CO_2 of NR or CO_2 of OR. When this is achieved it is brought out that the form of the glycolysis line is much simpler than that of the CO_2-production line. This is because it succeeds in presenting us with that greatest common measure of respiration, be it NR or OR, which we set out to seek in our analysis.

In proceeding from the CO_2 line to the glycolysis line we were working our way backward, by analytical treatment, from observed results to hidden cause. If, having attained a comprehension of the glycolysis line we now turn round to contemplate the devious course of the CO_2 line we see how its distortions reveal the magnitude of effects of post-glycolytic phases in the catabolism, phases in which, according to the oxygen supply, oxidation of the products of glycolysis D, may be either complete, partial or entirely absent.

For each analysed record, we shall have to give the quantitative relations upon which we have based our construction of the glycolysis line. The general nature of these numerical relations may be set out here so that only the values need be stated for each record. For the evaluation of glycolysis while in nitrogen we take it that three times the values of NR gives us an accurate measure, provided the rate of CO_2-escape is properly adjusted physically to the rate of NR CO_2-production. For the first 8 hours or so after entry into nitrogen we have a transitional phase, and the physical CO_2 lag conceals the full rate of NR. The initial NR, which is a value of vital analytic importance, is obtained only by extrapolation to zero hour. Glycolysis at the moment of transition is 3 × initial NR: this product must measure also the glycolysis rate in the oxygen mixture before nitrogen. From the numerical relation of the final OR value to the initial NR we get the ratio which enables us to determine the magnitude of glycolysis in air for that particular case.

$$\text{If } \frac{\text{initial NR}}{\text{final OR}} = 1\cdot 50, \text{ and Gl} = 3\text{NR, then Gl} = 4\cdot 5 \text{ OR}.$$

On the return from nitrogen to air we get another opportunity of checking the value of this ratio with lower absolute values of NR and OR, by the ratio, final NR/initial OR. In this situation, it is the precise initial value of OR which is masked by the CO_2 physical lag, now working in the opposite direction, and we again depend upon extrapolation to zero hour of the rising OR series.

In Table I we have set out the data for all the cases of nitrogen respiration examined in the previous paper. The second column contains the NR and OR values adopted at the change into nitrogen, while column 5 gives the resulting transitional ratios The ratios of Gl to OR will be threefold these ratios Column 4 contains as denominators the values of initial OR on passing out of nitrogen, which are indicated by the application of the adopted ratios

Table I —Table of Transitional Values and Ratios

Apple	Init NR / Fin OR	Hours in nitrogen	Fin NR / Init OR	Ratio	Class
XIX	22 0 / 14 2	70	1 0 / 11 0	1 55	A
XXI *a*	18 85 / 12 4	48	14 9 / 9 8	1 52	A
XXI *b*	14 65 / 9 7	36	9 3 / 6 2	1 51	A
XI	[16 40] / 10 9	40	14 5 / 9 7	1 50	A
XXIII	24 0 / 18 5	50	15 0 / 11 5	1 30	B
XXV	16 6 / 13 5	0	11 / 8 6	1 33	B
VII	20 4 / 13 0	70	11 1 / 4	1 50	C
XXII	21 3 / 16 1	0	12 7 / 9 6	1 32	C
XXIV	27 0 / 20 8	112	Killed	1 33	C

Initial NR values obtained by extrapolation Final OR and final NR values observed Initial OR values calculated by the initial transitional ratio and fitness judged graphically

The value of the OA component is evaluated from the same data being in terms of carbon units Gl less OR in any oxygen mixture and in nitrogen Gl less 3NR this latter being by our postulates zero Finally we can state the ratio OA/OR for each case

Apples of Classes A and B We may begin our survey with the apples of the Classes A and B which have already passed into their late starvation stage and consider first those only involving air and nitrogen

Records XXI, *a* and *b* see fig 3 On the falling CO_2 production line in air we have as final OR value 12 4 With entry into nitrogen we get a steep rise in CO_2 to reach the falling NR line which line by extrapolation gives initial NR = 18 85 The transitional ratio for air to nitrogen is therefore established

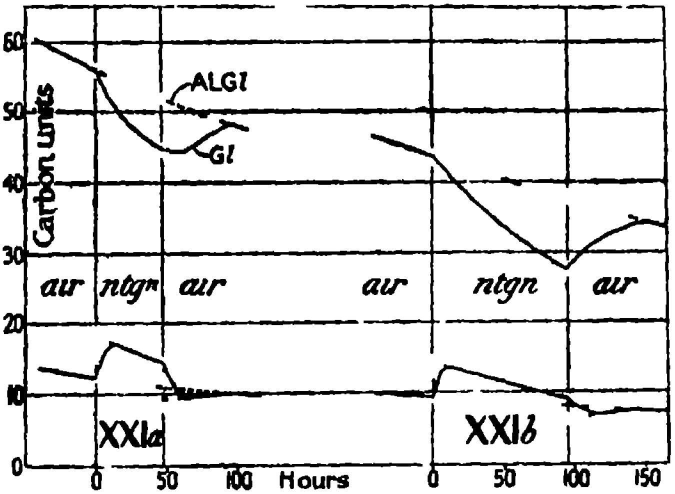


Fig 3 This and the next two figures illustrate the construction of the glycolysis lines from the CO_2 records Here the ordinate values are carbon units so as to give a possible common measure for CO_2 output and glycolysis The unit is the amount of carbon in 1 mgm of CO_2 i e 0 27 mgm C The ordinate numerals are therefore the same as those for CO_2 in the general records The lower part of each figure gives the rate of CO_2 production treated as in figs 1 and 2 while the upper part gives the evaluated glycolysis lines Gl being a continuous line for the actual glycolysis and AL Gl a broken line for the air glycolysis line which is the counterpart of the ALR for respiration

as 1 52 At the end of nitrogen NR has fallen to 14 9 which if the same ratio holds would indicate 9 8 as the initial OR value This value joins up well enough with the observed sequence of rising OR values so we regard the ratio as sufficiently established and are prepared to draw the glycolysis line at 3 × 1 52 OR for all times when the apple is in air When in nitrogen Gl = 3 NR so we can carry the line right through the record This glycolysis line Gl appears in the upper part of the figure as a continuous line in connection with the broken line representing the glycolysis air line AL Gl

Turning to the second nitrogen experience of the same apple XXI *b* some 130 hours later we find final OR = 9 7 initial NR = 14 65 giving thus a CO_2 ratio for air to nitrogen of 1 51 At the end of 100 hours in nitrogen NR has fallen to 9 35 which on applying the same ratio indicates an initial OR of 6 2 which takes its place perfectly as the start of the rising OR values making back to the air line We have then four evaluations of this ratio for one apple at remote periods of time namely at hours 48, 95 234 and 334 from the time of removal from store at 2·5° C. to the experimental chamber at 22° C. We consider it established by this record that this fundamental ratio is practically constant for a given apple and not subject to serious time drift. The ratio once ascertained for a given apple we can then use OR as a satisfactory index of glycolysis and its drift.

We may recall that this glycolysis line is not really a respiration line at all; it is a line of drift of carbohydrate catabolism. Its drifting form reveals that nitrogen depresses it;—by retarding the rate of production of the substrate C from A—B, according to our interpretation of the situation. When air is readmitted the rate of this production rises, and goes on rising till the production and consumption are balanced once more along the old line of starvation drift ALGl. This recovery of glycolysis rate takes about 45 to 50 hours in both cases and exhibits a simple transition. But when we look from the glycolysis transition to the identical transitional state on the CO_2-record in the lower part of the figure we find a triple complex transition, in which the drop of CO_2-production from Gl/3 in nitrogen to Gl/4·5 in air is very quick; while the transition of physical escape of CO_2, which is of moderate duration, also comes in to mask the course of metabolic events in the manner set out in the previous section.

With regard to the transition into nitrogen from air the CO_2-record shows an inverse triple complex transition, but from it there emerges the simple transition of the glycolysis line. We are, however, not able to state when this transition comes to an end, as the line curves down still after 45 hours and passes over into what we describe as a "semi-adjusted" course.

Let us pause for a moment to consider what the curve of *carbon loss* for these cases would be, though we have not drawn this curve in the figures. Its course presents no subtleties since in air it is identical with OR, being just the carbon contained in the escaping CO_2, while in nitrogen it is identical with Gl, the whole carbon of D being lost, either as alcohol or CO_2. At the transition to nitrogen the carbon loss shoots up from being identical with the lower record to become identical with the upper, where it remains until air is readmitted; after which it drops back to the lower again. Such a record would serve to bring out the enormous increase of loss that at once takes place in nitrogen, but it is not yet clear whether this great loss is a factor in causing the continuous rapid decline of glycolysis in nitrogen. According to the views already elaborated, this big loss comes about by nitrogen cutting off the formation of OA at the same time as OR, so that there is nothing produced in nitrogen which can be built back by anabolism to the carbohydrate group. We must therefore recognise the big part that oxygen plays in restraining carbon loss, when the state of things in air is compared with the state revealed in nitrogen.

The measure of the large amount of OA that is held to be formed in air is easily estimated by eye without an independent line since it is always Gl less OR. In these records OA is 3·5 times OR.

Record XIX associates itself very closely with these two, so much so that it might almost be an earlier nitrogen experience of the same apple. It is, however, only a short record, with no complete recovery to air after nitrogen, so that it will not be figured here. Also it must be borne in mind that its NR course has been very much smoothed (see 'A.S.R.,' II, fig. 3). For XIX the last value in air before nitrogen was OR = 14·2 while the extrapolated initial of the smoothed curve was NR = 22·0, giving a transition ratio of 1·55. After 70 hours in nitrogen the last NR value is 17·0, so that by the same ratio the initial OR after nitrogen should be 11·0, which is not out of harmony with the imperfect record, cut short by failure of temperature control. We conclude that glycolysis is 3 × 1·55 OR in this case.

Record XXV is that of an apple which has been assigned in Paper I of this series to Class B, on the evidence of the pitch and drift of its air-line. It provides a single nitrogen experience, 50 hours long, see fig. 5, p. 518. The final OR value before nitrogen was 12·5, and the initial NR 16·6, which gives us a ratio of 1·33. Later, the final NR was 11·5, indicating, by the 1·33 ratio an initial OR of 8·6. The transition air-to-nitrogen is a typical triple complex one, but the transition from nitrogen to air is an example of the quadruple transition that we described in the section on transitions, for here there is an additional feature of a small but definite after-effect (see p. 508). This hinders us from getting clear support for the suggested transitional ratio at the end of nitrogen, but OR = 8·6 serves well enough as an initial value. The glycolysis line for this B apple is constructed from the values Gl = 3 × 1·33 OR and Gl = 3 NR. The decline of glycolysis in nitrogen is seen to be much steeper in this record. Here OA, calculated by Gl less OR, has the value of 3·0 OR. We suggest that the 1·33 ratio is possibly characteristic of Class B as opposed to the group of ratios about 1·5 found for Class A.

Record XXIII is also for an apple assigned to Class B. It presents a new experimental feature in that the apple was for a long time in pure O_2 before experiencing nitrogen, see fig. 4. On passing from air to oxygen the CO_2 rises smoothly, at first steeply and then slower, till a crest is reached at 45 hours, at an OR value of 19·0 which is just 1·4 times the ALR value of 13·8 at that time.

Then for 30 hours the OR declines maintaining the same ratio to ALR constantly. Here then we find the adjusted pure oxygen line as a constant ratio to the air-line. This rise of CO_2-production produced by increase of oxygen has quite a different form of transition from the rise of CO_2 of about the same magnitude produced by nitrogen. It is of fundamental importance to ascertain whether glycolysis has gone up with the rise of OR, or whether this increased OR is due merely to heightened oxidation of D, the glycolysis remaining the same as in air. The nitrogen experience which follows settled this point quite definitely, for extrapolation of the NR series gives initial NR = 24·0, the highest value ever recorded; while the last OR value was 18·5, indicating a transitional ratio of 1·30, which concords with the ratio of the other B apple XXV.

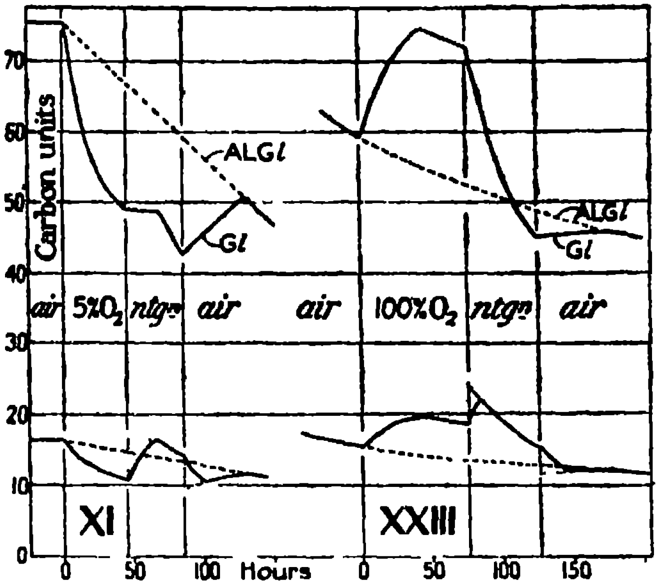


FIG. 4.—General description as in fig. 3. Records of XXIII and XI each begin with a simple transition followed by two of the complex type.

We note that at the time of transition the value on the ALR line is 13·2, which gives a ratio for NR/ALR of 1·82. It is this ratio that attention was focussed on in Part II of the previous paper, following the usual tradition; but now we learn that when the apple is in oxygen glycolysis is not the same as in air, and the nitrogen/air ratio loses any individual significance, being only the product of the OR/ALR ratio of 1·40 and the NR/OR ratio of 1·30.

Clearly glycolysis at the transition is 3 × 24·0 and has risen *pari passu* with the rise of OR, maintaining the appropriate 1·30 ratio. We require yet confirmation of the 1·30 ratio as specific for this apple and this we get at the transition from nitrogen back to air. The final NR value is 15·0 and the form of the rising OR values confirms the initial OR value of 11·5, which the 1·30 ratio would indicate if it held throughout this apple record. We conclude then that, however high the value of OR may be forced, glycolysis rises in the same ratio continuing to be evaluated by $Gl = 3 \times 1\cdot30$ OR.

In thus speaking of OR as if it were a determiner of glycolysis we must realise that we are inverting the real sequence of causation for we regard OR merely as an index and not a cause of glycolysis. Also glycolysis, in its turn is taken to be only an index of the production of C. If our views were absolutely established our statement would take the form that pure oxygen has increased the production rate of C to 1·4 the value in air, and that the rates of glycolysis and OR rise consequently in the same proportion, OR continuing to be one quarter $\left(\frac{1}{3} \times \frac{1}{1\cdot30}\right)$ of glycolysis all along. Hence $OA = 3\cdot0$ OR as contrasted with $OA = 3\cdot5$ OR for Class A.

All glycolysis transitions are, by definition, simple in form and slow in progress but with this apple on passing into pure oxygen from air we have met for the first time an OR transition which is also simple and slow. This is confirmatory of the view that the complex form of transition is to be attributed to the intervention of NR, causing a shift of the post-glycolytic relations.

This record contains the most striking variations of glycolysis rate that we have yet met, and we note how steeply the rate falls off in nitrogen after the height to which it has been pushed in pure oxygen.

By giving oxygen to raise glycolysis, and then nitrogen to lower it, it is possible to meet a moment of time in nitrogen at which the glycolysis rate, being still unadjusted just happens in passing to attain the value appropriate to air at that time. This is located in the figure at the moment when the falling glycolysis just cuts across the line that glycolysis in air would have followed. According to our present views, return to air at that moment should arrest Gl at that value and maintain it there. The observed CO_2 would be expected to drop from $NR = 1\cdot30$ OR to OR and stay there without dipping below ALR and creeping up again. This would be an interesting region for future experimentation; for here a change in oxidation might be examined apart from any change in glycolysis rate.

There is still one record left to be considered, apple XI, also presented in fig. 4. This differs from all the others in the fact that it is an apple in the early stage of storage and not in the late starvation stage as the other five. In the first paper the characteristics of the early phase are fully discussed, but

A and B in the opening pages of the previous paper, and at first sight the classes appeared to have no feature in common. It is perhaps significant that it was on the evidence of the course of the respiration in air that this class was set apart in the first paper of this series. That course is a result of carbohydrate metabolism and of the supply of substrate for respiration. Since we have now come to the conclusion that the oxygen-nitrogen behaviour of apples is primarily an outcome of the effects of these gases on the carbohydrate equilibration, which controls glycolysis, it is no longer surprising to find that a class which shows a special starvation behaviour in air should also show special features in nitrogen. Taking up the method of analysis that has been worked out for Classes A and B we now find that it clarifies the nitrogen behaviour of Class C to such an extent that we can now regard this as a special case of the general formulation and no longer a type quite apart.

The three apples of this class that were subjected to nitrogen bore the numbers VII, XXII and XXIV, one being very early in the storage series and the other two very late. The transitional features of VII and XXII are to be found in fig. 2, and the glycolysis line of VII in fig. 5. provided with construction lines of the type we have already described. The special features of these apples that arrested us, on empirical survey, were that the CO_2-production in nitrogen is identical with that in air, for a considerable period of time, and that then, quite suddenly, NR drops away rapidly. Added to these two features was the appearance of a special transitional effect in that the CO_2

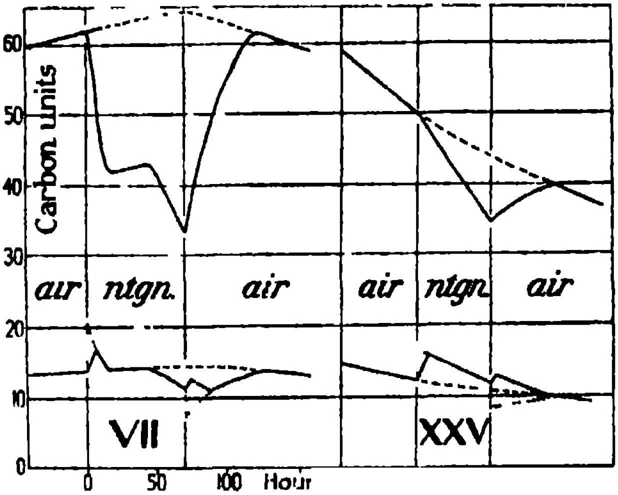


Fig. 5.—General description as in fig. 3. Record XXV has been discussed earlier (see p. 514).

output rose at first for a few hours in nitrogen and then fell back to become again level with the OR values before nitrogen. This feature was at first put aside as some obscure disturbance of CO_2-production at the cutting off of air, but now that we have elucidated the components of thec omplex transitions of Classes A and B we acquire the key to the significance of the form in C. It seems reasonable to interpret the initial form which gives a rise of CO_2 for some 8 hours followed by a steep fall, as being really the expression of a high initial NR falling steeply away, distorted by physical lag in CO_2-escape from the apple. The magnitude of the initial NR was therefore to be arrived at by extrapolation of the observed falling slope back to the origin and a value obtained for comparison with the final OR. We could thus evaluate the ratios which gives a measure of the rate of glycolysis in air, see Table I, p. 511.

Record VII treated in this way gives us an initial value of NR = 20·4, while the final OR was 13·6. We get from these the same ratio of 1·50 that we got for Class A apples. We conclude that here also glycolysis = 3 × 1·5 OR while in air. This ratio is supported fairly well by an examination of the ratio at the end of nitrogen where the final NR is 11·1, indicating initial OR of 7·4. The evidence cannot be decisive here because in this apple as in the other apple of Class C there is superposed on the complex triple transition a small after-effect of exactly the type described for apple XXV.

On the sum of this evidence we may construct the glycolysis line of VII as equal to 4·5 OR in air and 3 NR in nitrogen Clearly the form of this line in the upper part of fig. 5 is not fundamentally different in its gas relations from that of Classes A and B, but it does present a special secondary difference in this, that instead of falling away in nitrogen along a smooth curve from the initial it first falls fast and then maintains a short level phase for a period of time before the fall is continued, rather suddenly and rather steeply. On our view this behaviour must be an expression of the factors governing the supply of the glycolysis substrate C. It is a difference of carbohydrate metabolism and not of respiratory oxidation. The relations of Gl to OA and OR, and of Gl to NR are the same as in Class A. The characteristic thing is the form and rate of the depression of glycolysis on continuance in nitrogen. Whereas, with apples of Class A, glycolysis in nitrogen is depressed in a smooth falling curve at such a rate that NR, which equals Gl/3, never gets as low as OR in the experimental period, here in Class C we find that 15 hours suffices to depress glycolysis so that Gl/3 is just equal to ALR, while after 45 hours this value falls away fast to below ALR.

The rapid decline observed in these apples seems associated with a rapid power of recovery of glycolysis in air for after nitrogen the original rate is built up again in about 45 hours in spite of the very great previous depression

This form of the depression of glycolysis which appears as exceptional among our apples occurring only in association with those apples that can keep up a level respiration in air has been found by our investigation of other plant tissues to be the typical form in leaves of Cherry Laurel In these leaves when starving carbohydrate metabolism runs a different course from that of starving apples as shown by their records of respiration in air and in them nitrogen invariably produces exactly in every detail the form of effect here described for apple VII The significance of this form will be taken up again in our analysis of the respiration of these leaves in a future paper

The two other apples of this class must be briefly examined Apple XXII agrees exactly with VII in type but can maintain the level middle period in nitrogen only for a short time the rectilinear fall sets in after 22 instead of 45 hours Its transitional behaviour appears in fig 2 but its glycolysis features agree so well with those of VII that no figure is presented The extrapolation of the first falling NR values points to an initial NR value of 21 3 which gives a ratio of 1 32 to the last OR value of 13 6 before nitrogen The glycolysis line would therefore be drawn as Gl = 3 × 1 32 OR At the end of nitrogen we find final NR = 12 7 indicating by this ratio an initial OR of 9 6 This fits in well enough with the rising curve of OR values reaching the air line in about 45 hours but the situation is obscured as in VII by the presence of an after effect

Apple XXIV gives a fragmentary record which is therefore not figured except in the general records It was not returned to air after nitrogen as we wished to follow the nitrogen depression which here was associated with toxic effects The early fall of NR is very steep and the extrapolation to the origin is not very securely located but we accept 27 6 as the initial NR This gives a ratio of 1 33 to the final OR of 20 8 The glycolysis line would therefore be constructed at Gl = 3 × 1 33 OR

In record XXIV the level middle phase in nitrogen is so short on the NR record that its existence might have been ignored had it not been for the indications of the records of VII and XXII The decreasing duration of this middle phase along the chronological series we should attribute to altered carbohydrate metabolism with metabolic drift We may note that in apple XXIV the form of the NR progress has become practically a continuous curve, which brings it within the formal definition of Class A though very much steeper

Surveying these ratios for the three Class C apples we note that VII gives 1·50, while XXII gives 1·32 and XXIV 1·33. We find then both types of ratio in one class, but it must be mentioned that seven months elapsed between VII and the two later examples. There is possibly a correlation of the lower ratio with late metabolic states.

SECTION V.—CONCLUSION: THE OUTSTANDING FEATURES OF THE PROPOSED RESPIRATION SCHEMA.

In the first section of this paper we set out dogmatically a proposed schema of respiration reactions; in the second section we sketched our view of the functional working of this chain of reactions in relation to starvation and to variation of oxygen supply; in later sections we turned to the actual records, and demonstrated that the system provides a plausible interpretation of all the quantitative variations of CO_2-production that we had observed in these apples.

Now that we have worked through all these particular aspects of the matter we may conclude by surveying more generally the essential features of the new situation. The most fundamental departure is that attention is concentrated upon the rate of glycolysis, as much when the tissue is in air as when in nitrogen. Glycolysis is regarded as the common measure of respiration in all conditions. The production of CO_2 provides us with an index of the magnitude of glycolysis.

Another feature is the adoption of the view that normal hexoses (group B of the schema formulated on p. 492) are not the direct substrate for glycolysis or oxidation but that some specialised derivative of them is indicated for this function. This we represent by our group C, suggesting that the more active heterohexoses might prove a suitable representative for this position. The general reasons for interpolating a new reactant in the series are based on dynamical considerations. The way the apple metabolism responds to changes of oxygen supply suggests that the significant reactant is not a substance of high concentration which can undergo only slight alterations of amount, but rather one of low concentration which is subject to marked changes of concentration, production, and consumption within the range of the experimental changes we have imposed upon our apples.

It is clear that we can alter the glycolysis rate from a minimum of unity to a maximum of threefold. Postulating that the mass laws hold in this metabolism, at least a similar shift in the concentration of the substrate is required. We have no estimations of carbohydrates available for these apples, but general knowledge of sugar content gives no support to the view that the amount of normal hexoses is sufficiently mobile. Exact knowledge in this field is being rapidly acquired by direct and continuous analytic studies of sugars in apples carried out by other workers in connection with the Food Investigation Board; and from this survey it should be possible to make a final decision by combining carbohydrate analyses with direct respiration estimations.

In suggesting that the concentration of a substrate C, of which we have no knowledge in apples, is really the important aspect of all types of respiration, we have simply followed up the indications that we draw from our data. We have data from other tissues awaiting similar analysis, so that later we shall see where they in their turn appear to lead us. After that it may become necessary to take the present schema to pieces and reconstruct it, but at least we shall have consolidated a mass of relations to which any future system must conform.

Another new feature which arises out of the analysis of apple respiration and the concentration of attention on the rate of glycolysis is that in air a large amount of some substance, which we have labelled OA, is formed concomitantly with the $CO_2 + H_2O$ of OR. This conception, again, arises out of the facts presented to us and seems unavoidable, but as to the exact status and fate of OA we have not yet any decisive evidence. That it does not accumulate seems certain, and so we consider it as being built back into the stream of catabolites. It would be possible to hold that OA results from a catalytic oxidation process, which is independent of OR but has identical oxygen relations, or that OA is an antecedent of OR, so that in air the whole, of glycolysis goes to OA first, while only part of it is oxidised on to CO_2. If the concentration of OA were low with a specific catalyst converting it to OR, then a constant relation between production of OA and OR might still be maintained through considerable variations of the rate of total glycolysis. Even within the range of variation of metabolic types presented by this one lot of apples we find evidence that the ratio of OA to OR may range from 3·0 to 3·5, when types are contrasted, though it seems to remain constant within a given type. With other plant tissues we may find such a variation of this ratio that the production of OA and of OR will cease to be regardable as two colligate aspects of one catalytic activity.

In spite of the uncertainties that surround the new components C and OA, introduced into our survey of respiration, we hold that one definite advance has been made by showing how glycolysis in oxygen mixtures can be evaluated, and a second by reaching the conclusion from such evaluations that glycolysis proceeds at a greater rate in air than in nitrogen, and is still further accelerated by further rise of oxygen concentration. It is here suggested that this acceleration of glycolysis by oxygen is not due to oxidation but to the acceleration of the rate of production of the substrate for glycolysis. Should it be established that the primary effect of varying oxygen supply in respiration lies in the control of carbohydrate equilibrium, then our biological outlook on this function will be considerably modified.

A Study of the Adrenal Cortex in the Mouse and its Relation to the Gonads.

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[Plates 11-15.]

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Introduction.

In 1920 Masui and Tamura published a study of the adrenals of mice (20), and drew attention to the larger size of the female adrenal as compared with that of the male. They state that this is due to the degree of development of the zona reticularis, which is reduced to a few cells in the male, but may be of considerable size in the female. The extent of the zone in the female, however, varies greatly; it disappears almost entirely in old animals. After castration, the zona reticularis grows in the male and the gland tends to reach the female size; ovariotomy has no effect on the adrenal. The statement is made (p. 373), that "the size of the adrenals shows a considerable difference according to various periods of the œstrous cycle, and this variation in size is due to the structural difference of this gland." During pregnancy and lactation the glands are said to diminish in size, but the weights are not given.

Shortly afterwards Tamura published a further paper (25), on structural changes in the adrenal during pregnancy. He states that the gland becomes reduced in area owing to the degeneration of the zona reticularis before the formation of the placenta; the two outer zones of the cortex increase in size during pregnancy, as does the medulla.

Subsequent research has only partially substantiated the conclusions of these writers; their tables of measurements are of doubtful statistical significance.

The present work was undertaken in order to ascertain whether variations in the adrenal cortex could be correlated with phases of the œstrous cycle, as Masui and Tamura had suggested; it was later extended to the adrenals of both sexes and their relation to the gonads. The experimental work was almost completed before an important paper (21), on the same subject appeared. It was then found that Mrs. E. Howard-Miller had already made a very similar study of the adrenals of mice, and had arrived at conclusions agreeing closely with those recorded here concerning the histological changes which occur in the cortex. The present account is designed to confirm and supplement Miller's observations, and reference should be made to her paper for further details.

Miller describes the differences between the adrenals of male and female mice, and traces the changes taking place in the course of the life-history. The large inner cortical zone, present at puberty in the unmated female, and absent from the male, is termed the X zone; ending further comparative work, Miller prefers not to call it the *zona reticularis*, under which name it was described by Tamura (20, 25).

The X zone is said to disappear normally from both unmated and mated females during the first half of the reproductive period, the time of its disappearance varying very considerably. The sequence and time relationships of the degenerative process were studied by means of unilateral adrenalectomy, followed after intervals of 1 to 21 days by removal of the second gland. The histological process of degeneration is described in detail; no reference is made to recrudescence of the X zone In the unmated female there was found no correlation of the œstrous cycle with changes taking place in the adrenal cortex Pregnancy accelerated the degeneration of the X zone but had no specific effect on the histological structure of the gland The statements of Tamura and others (1 20) with regard to the effects of gonadectomy on the adrenal are corroborated by Miller

Material and Technique

The mice used in this work belonged to a colony maintained at University College by Dr A S Parkes details of its management have been given in a previous paper (22) Owing to the liability of adrenalectomised animals to suffer from cold special efforts were made to secure a constant suitable temperature but otherwise these mice were maintained on a balanced diet under the same conditions as the rest of the colony

Dr Parkes has carried out all the operations in connection with this work and has also been responsible for observations on the œstrous cycle and breeding I am deeply indebted to him for his constant assistance

Operative Technique —The mice were anæsthetised and the adrenals removed without ligaturing through a slit in the dorsal body wall Ovariotomy was performed in the same way but the vessels were ligatured Castration was effected by means of a mid abdominal incision not through the scrotum

Histological Technique —Three principal fixatives were used for the adrenals in this work Bouin s fluid for micro anatomical detail Flemming s fluid (with acetic) for the fixation of fatty substances and Ciaccio s fluid* followed by Sudan III for lipoids

The glands were imbedded in paraffin and cut in complete serial sections For the study of fats and lipoids sections were mounted in Farrants glycerine medium Among the stains employed were iron hæmatoxylin followed by Van Gieson s picro acid fuchsin and Ehrlich s hæmatoxylin with eosin or Pasini The latter served to differentiate the fibrous reticulum and the cytoplasm of different parts of the cortex

It is realised that the methods listed above can only give approximate indications of the nature of the cell contents it seems possible however with their aid to distinguish differences in the cell contents in various parts of the gland and at various times In the following histological descriptions the term fats is used for substances which are blackened by osmic acid and lipoids for substances stained brown with osmic acid or orange with Sudan III after fixation in Ciaccio s fluid

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* Ciaccio s fluid —

5 per cent potassium bichromate	16 c c
40 per cent (concentrated) formol	4 c c
Glacial acetic	1 c c

I.—*General Description of the Mouse Adrenal.*

Size of the Glands.—Maximum horizontal sections through glands from young adult male and female mice are shown in Plate 11, figs. 2 and 3; these illustrate the sex differences in the size of the adrenals. A comparison of the weights of the glands in six males and four females indicates that the female gland weighs approximately three times that of the male, but both show considerable variation in size. Miller (21) finds that in the male the left adrenal is about 10 per cent. larger than the right. In my own material female glands may be found belonging to the same animal which show a very great discrepancy in size; one gland may be four times the size of the other, the smaller occurring on either side of the body. A considerable size difference may also exist between the two glands of a male. These variations in apparently normal animals are so great that estimations of hypertrophy (*e.g.*, following unilateral adrenalectomy or during pregnancy (26)) would be very liable to error, unless calculations were made for a large number of animals. Observations on glands cut in complete serial sections indicate that compensatory hypertrophy in the remaining adrenal following the removal of the other is not noticeable until more than three weeks have elapsed after the operation. Fig. 7 shows a section through an adrenal seven weeks after the removal of the one on the other side.

Histology.—The two adrenals of an animal, even when differing in size, are always found to be histologically similar. This is in agreement with the statements of Miller (21) for the adrenal of the mouse, and of Guieysse (8) for the adrenal of the guinea-pig.

Figs. 2 and 3 illustrate the structure of the glands in male and female mice of the same age. In both the edge of the cortex is occupied by the zona glomerulosa, where the cells are flattened perpendicularly to the radius, and arranged in rounded masses; below this lies the zona fasciculata, consisting of regular radial strands of cells. In the male gland this passes over imperceptibly into a region varying in size, but often small, where the cells are more or less irregularly arranged and have slightly darker staining cytoplasm (fig. 9); these cells are similar in size to those of the zona fasciculata in most males, but sometimes smaller, as in the corresponding region of the female gland (fig. 2). Among these inner cortical cells runs a well-marked band of fibrous reticular tissue, forming an apparent medullary boundary in the male gland; this is absent from the gland of the young adult female (fig. 3). This "medullary membrane," as it has been termed, has been referred to by numerous writers on the adrenal, and is often described as consisting of fibrous connective tissue. Flint (7) and Corner (5), however, claim to have shown that the framework of the adrenal consists of a "fibrillar reticulum," similar to the one in the liver and lymph glands. The staining reactions of this substance are similar to those of fibrous connective tissue, but the two can be distinguished by the Bielschowsky-Maresch silver-nitrate method. Corner maintains that the "fibrillar reticulum" develops from the endothelium of the capillaries, and this is in agreement with my own observations on the mouse adrenal. Further reference to the development of this fibrous tissue round the medulla will be found below.

In the young female, up to two-thirds of the adrenal cortex may be occupied by a dark staining zone, whose cells project irregularly into the medulla (fig. 3); the transition between the zona fasciculata and this zone, in which the cells are small, closely massed and more deeply stained, is normally abrupt. The dark inner zone is termed "zona reticularis" by Tamura, and "X zone" by Miller. In older females, the X zone disappears partially or completely and the cortex of the adrenal becomes histologically similar to that of the male, though the size difference in the glands persists (fig. 5). Pigment is absent from the adrenal cortex in mice.

The Nature of the Zona Reticularis. - A study of a number of adrenals from different mammals makes it apparent that the zona reticularis of the cortex is by no means a clearly definable region. For the present, however, the term may be applied to the inner part of the cortex where, normally, blood vessels and fibrous tissue are prominent, and the cells show a reticulate or irregular arrangement.

In all rodents examined, other than mice, a zona reticularis may easily be distinguished in the adrenal; there are no obvious histological differences associated with sex, though the gland of the female rat is larger than that of the male (9, 11).

If a number of adrenals from male mice—particularly glands fixed in Flemming's or Ciaccio's fluids—are examined, there can be no doubt that there is an inner region of the cortex which corresponds to the zona reticularis of other mammals (figs. 2 and 9); compared with the rest of the cortex, this area in the mouse is practically free from fats. In the latter respect it resembles the large X zone in the young female, and also the inner part of the cortex after the X zone has degenerated.

It seems reasonable to regard the X zone at its maximum in the young female as a transitory development of the zona reticularis, which is much smaller in the adult animal.

Tamura (20) states that the zona reticularis has disappeared almost entirely from the male mouse adrenal; he bases this view on the fact that at about four weeks old, the dark staining zone present in both sexes next the medulla (fig. 1) apparently degenerates in the male, whereas it continues to grow in the female and forms the zona reticularis (X zone). The explanation seems to be that some cells degenerate, and the remaining ones do not increase comparably to the rest of the gland; the area they occupy is sometimes so small in the adult male adrenal as to be readily overlooked, particularly if the fixative used has not preserved the cell inclusions.

In the present paper, Miller's term "X zone" will be retained for the conspicuous development of the zona reticularis present in the young female mouse, in order to distinguish it from the corresponding small region in the glands of adult mice of both sexes.

II.—*The Development and Histology of the Adrenal in the Male.*

Thirteen glands were studied from mice 19–35 days old and fourteen from older males. At three weeks the cortex of the adrenal has not yet developed its adult characteristics; the cells show no regular arrangement and have not yet attained their full size. The inner part of the cortex is closely interlocked with the medulla and differentiated from the rest by the smaller size and more darkly staining cytoplasm of its cells (fig. 1). This region appears to be comparatively free from fats and lipoids.

In the course of the next two weeks the adrenal grows and approximately reaches its adult size; the growth appears to be brought about more by an enlargement in the size of the cells than by an increase in their number. The darkly staining inner zone practically disappears during this time. Its suppression seems to be connected with the early cessation of growth in the male gland as compared with that of the female. Castration experiments show that the male adrenal is influenced by the testis during this period.

Examination of sections through a male gland where the inner zone is in process of disappearance fails to reveal any obvious amount of cell degeneration, but an increased prominence of reticular connective tissue can be observed round the medulla. Gradually this fibrous tissue forms a complete ring, and some of the cells in the darkly staining zone which remain inside it are pushed into the medulla (fig. 8). Since cortical cells cannot be found in the medulla of fully developed male glands, they must be presumed to degenerate; just prior to their disappearance, these cortical cells contain small globules of fats and lipoids which were absent at an earlier stage.

Some of the dark staining cells of the original inner zone persist outside the ring of fibrous tissue these form the irregular zona reticularis round the medulla distinguishable in the glands of adult mice (figs 2 and 9) This can be readily seen in large adrenals but in small ones it is poorly developed and inconspicuous Proportionately to the rest of the gland it is smaller and less distinct than the darkly staining zone visible at three weeks Fat is absent from the zona reticularis in the adult gland but lipoids occur throughout the cortex, though they are commonly more abundant towards the exterior

III —*Development and Histology of the Adrenal in the Unmated Female*

In the course of this study 40 glands were sectioned from animals known to have been unmated these form two series The first comprises 8 animals of known age from 3 to 12 weeks whose glands were sectioned in order to trace their normal growth and development The second series consists of 14 mature females (over 8 weeks old) whose exact age was not recorded To these adrenals may be added the left glands of the animals described in Section IV

The changes occurring in the adrenals of unmated mice will only be referred to briefly here as they have been fully described by Miller (21) At 3 weeks old the adrenal of the female resembles that of the male at the same age (fig 1) The transition between the darkly stained inner region and the rest of the cortex is not so abrupt as in the older female where the X zone is fully developed During the time when the inner zone is disappearing from the male gland it is growing rapidly in the female and by 5 weeks old it occupies about half the cortex Its cells* which are smaller than those in the zona fasciculata ramify among the medullary tissue and can be seen forming darker groups among the lighter staining medullary cells There is no band of reticular connective tissue round the medulla such as has developed in the gland of the male mouse of the same age In rather older females isolated groups of cortical cells in the medulla can still be found but are less common The proportionate size of the X zone appears to be independent of the size of the gland some adrenals which are below the average and hardly larger than those of a male show a well developed X zone The present material does not indicate at what average age the X zone begins to break down in the unmated female contrary to Miller s statement however it may be still intact in a 12 weeks mouse (fig 3)

* Lipoids are present in the X zone at this stage while growth is continuing but are absent from the fully developed zone though they reappear when degeneration sets in

Histology of Degeneration.—The adrenals of the second series, consisting of mature unmated mice, are referred to below in connection with observations on the œstrous cycle. The types of gland occurring among them are illustrated in Plates 14, 15, figs. 11–15. They confirm Miller's account of the normal degeneration of the X zone in unmated females. This author describes two modes of degeneration: either the greater part of the zone becomes gradually vacuolised (fig. 14), and degenerates *in situ* leaving a mass of reticular tissue enclosing large fat globules, or the cells merely become shrunken and crowded together, with little or no vacuolisation, and the X zone is thus slowly reduced in size, till it finally disappears.

Both these types of degeneration have been observed in the present material; in most glands studied, however, the X zone seems to disappear gradually as the result of an inconspicuous process of cell degeneration, which may begin either at the outside of the zone (fig. 12) or next the medulla. The space occupied by the degenerating cells is first filled with reticular tissue and capillaries distended with blood corpuscles (figs. 11 and 13), and later obliterated. Large fat vacuoles may occur next the medulla when the zone is more than half absorbed (fig. 13), but they are often absent in the earlier stages and do not form a constant feature of the degenerating zone. During the resorption of the X zone the reticular fibrous tissue, which appears to be derived from the endothelium of the capillaries, proliferates and becomes more prominent; eventually, when the zone has undergone considerable degeneration, this fibrous tissue forms a complete ring round the medulla (fig. 15); a few groups of cortical cells left inside this ring become pushed into the medulla and subsequently disappear. The fibrous band persists and forms a cortico-medullary boundary as in the male, where it has arisen earlier in the same manner. A remnant of cells from the original X zone may persist among the fibrous tissue and outside it.

The various types of histological degeneration are in no way mutually exclusive; a combination of all three -vacuolisation, crowding of cells, and increased prominence of the capillaries and reticular tissue accompanying progressive cell degeneration—may occur together in the same gland (fig. 13) or either of the first two types may be associated with the latter. The degeneration of the X zone in the unmated female appears to be a slow process; the pairs of glands removed from animals at intervals of 5 or 11 days showed no appreciable histological differences, even where degeneration of the X zone had set in.

In one case, the left gland was removed from a female and found to be extensively vacuolated (fig. 14); 3 weeks later the remaining gland was obtained and found to be histologically similar, showing that the vacuolated condition is sufficiently stable to persist for an appreciable time.

A gland from another animal contained an X zone that was still moderately large; this was degenerating, reticular tissue and capillaries being prominent, but fat vacuoles absent. In the second gland, obtained 3 weeks later, the X zone was similar, but not noticeably smaller. Miller's work (21) has shown that removal of one gland does not interfere with the degenerative process in the other, consequently it must be inferred that the process is commonly slow, in the absence of pregnancy.

Development of a New Cortical Zone after disappearance of the X Zone.—Miller records a considerable variation in the time of disappearance of the X zone from virgin females; the degenerative process is assumed to be irreversible. The present material, however, includes a small number of animals, in which an area similar to the X zone had developed round its fibrous remains. The interval which elapsed between obtaining the left and right glands was 4–7 weeks, the left being removed first. It was not known whether these animals had been pregnant prior to the operation, but in the time following unilateral adrenalectomy they were unmated. Complete degeneration of the original X zone had taken place in all these animals, leaving a fibrous zone round the medulla. It is believed that the new inner cortical zone is the homologue of the zona reticularis in other adult animals, and it will be described under that name.

SCM 11.—In the left gland the inner part of the cortex, adjacent to the fibrous zone, consists of undegenerating cells; in size and staining these are intermediate between the cells of the zona fasciculata and those of the X zone in the young female. The area which they occupy appears darker than the rest; it is not sharply defined but occupies between a third and a quarter of the depth of the cortex. The appearance and dimensions of the cells and the absence of prominent blood vessels, fibrous reticular tissue or vacuoles, distinguishes this irregular zone from an X zone in course of degeneration.

In the right gland, removed three weeks later, this zona reticularis is larger and more distinct at its outer edge, the blood vessels and fibrous tissue next the medulla, however, appear to be extending into it, and it is probable that degeneration of the type found in the X zone is beginning.

SCM 4.—No definite inner zone can be traced in the cortex of the left gland, but the cells next the fibrous zone are slightly darker staining than the others. The right gland was removed four weeks later, and shows a well-marked zona reticularis, where the cells are both smaller and darker staining than those in the rest of the cortex (figs. 6 and 17). There are no indications in it of hyperæmia, and the fibrous reticular tissue round the medulla has only penetrated the zone appreciably in one place.

SCM 13.—In the left gland traces of the degenerate X zone persist in the fibrous connective tissue; outside this the cells were smaller than in the rest of the cortex, but did not form a distinct zone.

In the right gland, removed five weeks later, there was a well-marked dark staining zone round the fibrous tissue, similar to the one which had developed in SCM 4, and occupying two-fifths of the depth of the cortex. This was extensively vacuolated and hyperæmic.

SCM 15.—In the left gland the inner part of the cortex is hyperæmic, but there is no zone of small cells. In the right gland (fig. 7) removed seven weeks later, a large irregularly shaped zona reticularis is present, of which the limits are quite distinct, owing to the difference between the size of its cells and those of the zona fasciculata. The inner part of the zona reticularis is hyperæmic and it contains a number of degenerating cells.

Less definite indications of the growth and differentiation of an adult zona reticularis were observed in the glands of two other females, removed at intervals of three and six weeks.

A study of the glands described above shows that the inner region of the adrenal cortex in the female may undergo changes after the original X zone has disappeared. Mitoses were very rare in the material examined, so that one cannot definitely exclude the possibility that the new zone is the result of an inward movement of the fasciculata cells, accompanied by proliferation in the glomerular layer or elsewhere. At the same time, there is no evidence of any such migration, and it seems more probable that the zona reticularis arises *in situ*, in the same manner as the zone forming in the gland of the male after castration.

The circumstances leading to these cortical changes in the female gland are unknown; they are not associated solely with compensatory hypertrophy, following the removal of one gland, and are probably unconnected with it. SCM 11 showed a zona reticularis in the first gland removed, which was apparently beginning to degenerate in the other gland, 4 weeks later. Marked degenerative changes in a zona reticularis in the right gland of SCM 13 also indicate its variable character.

It is possible that the formation of a well-marked zona reticularis only takes place in animals where the original X zone has disappeared early before the gland has lost the power of further growth.

The Œstrous Cycle and Adrenals in the Unmated Mouse.—Vaginal smears were taken daily in a number of unmated mice; a record was made of the phase of the œstrous cycle at which one or both adrenals were removed. In six animals both glands were obtained simultaneously, in nine an interval of 5 days elapsed between the removal of the left and right glands, and in three animals the interval was 11 days. In all cases the pairs of glands were histologically similar to each other. including those removed at different periods of the œstrous cycle. On the other hand, adrenals obtained from different animals at corresponding periods of the cycle were of all types.

It may be concluded that histological changes in the adrenal cortex of the unmated mouse show no relation to the œstrous cycle.

IV.—*The Effect of Pregnancy on the Adrenals.*

Twenty-eight adrenals were examined from 17 animals, in various stages of pregnancy and lactation; the exact age of the mice was not known in all cases. The observations on these animals may be summarised as follows:—Five animals killed when not more than 7 days pregnant showed various types of X zone; in a primiparous, 50 days old female, the X zone was intact and interlocking with the medulla, in the others the X zone was small and vacuolated, and a connective tissue band had already formed round the medulla. In 4 animals, killed when 11 to 16 days pregnant, only a remnant of the X zone persisted round the medulla; the glands were of the type shown in figs. 15 and 16. In 8 animals killed during lactation, the X zone had practically disappeared from the adrenals and there was no sign of its recrudescence.

These preliminary observations, together with those on the glands of unmated females, indicated the necessity of examining the effect of pregnancy under more accurately controlled conditions. Accordingly the left adrenals were removed from 24 females and sectioned and examined. A considerable amount of X zone was present in the glands of 17 of the mice; these animals were selected for mating as possessing adrenals likely to show the maximum changes as the result of pregnancy. Degeneration of the X zone, when present, had in no case proceeded far enough for the formation of a complete connective tissue band round the medulla, once this has taken place, cortical and medullary tissues no longer interlock.

Fourteen of the 17 animals selected for the experiment were mated with normal males 1 to 11 days after the removal of the left gland; all copulated and 10 became pregnant; in 4 cases in which the first copulation was infertile, the vaginal plug was found less than 2 days after the operation. (SCM 25, 26, 29, 30.)

The condition of the remaining adrenal in the series of mated animals and unmated controls is summarised in the tables below. A comparison of the right adrenal with the left from the same animal will indicate any changes caused by pregnancy; changes resulting from progressive normal degeneration will be indicated equally by the non-pregnant controls.

Table III includes all the animals whose left gland contained a large X zone, occupying more than half the depth of the cortex; among these indications of degeneration (vacuoles or increase in the reticular tissue), were absent or slight (fig. 3).

Table IV includes animals in which the X zone had already undergone some reduction, or showed well-marked indications of degeneration. In all these, except SCM 19 and 24, the zone occupied one-third to two-fifths the depth of the cortex, and fibrous tissue and blood vessels were more prominent than in the undegenerated zone of a young animal (fig. 12). SCM 19 had a left gland containing a large X zone, of which half the area in section was occupied by vacuoles (fig. 14); SCM 24 was of a similar type, but vacuoles only occupied about one-eighth of the area of the zone.

Table III.—Large Undegenerate X Zone in Left Glands.

No. of female.	Days after the operation.			Days pregnant (post coitum).	Condition of X zone in right gland.
	Mated.	Vaginal plug.	Killed.		
SCM 30	1	2	9	—	Similar to left gland.
SCM 27	—	—	14	—	" "
SCM 25	1	2 & 16	23	7½	Reduced to one quarter of previous size; remainder shows conspicuous schlerosis.
SCM 5	6	9	21	12	Reduced to a few cells among fibrous tissue.
SCM 17	11	14	26	12	" "
SCM 8	6	7	21	14	" "
SCM 9	6	8	26	18	" "
SCM 16	6	9	21	—	Similar to left gland.

The results summarised in this table indicate clearly that pregnancy causes a rapid and complete degeneration of the X zone, even if no reduction had been previously apparent. Copulation alone does not initiate degeneration, and the X zone may persist in the same histological condition for at least 3 weeks, following the removal of the other gland. In SCM 9, 17 and 25 the interval between the fixation of the right and left glands was only 2 to 5 days longer than in the non-pregnant animal SCM 16, in which no degeneration of the X zone was apparent; the striking changes in the right glands of the former are undoubtedly due to pregnancy and not to the additional days in the interval. The fact that fibrous reticular tissue appears round the medulla in the place of the X zone in all animals more than 10 days pregnant (fig. 16) makes it possible to state definitely from the condition of their glands that none of the 17 animals used in this experiment had previously been pregnant.

Table IV —Partially Degenerated X Zones in Left Glands

No of female	Days after the operation			Days pregnant (post coitum)	Condition of X zone in right gland
	Mated	Vaginal plug	Killed		
SCM 26	1	2	9	—	Has almost disappeared
SCM 29	1	2	9	—	Reduced to one half of previous size
SCM 18	11	14	21	7	Similar to left gland, but rather more vascular
SCM 24	1	6	13	7½	Size similar to left but four fifths of area vacuolated
SCM 19	—	-	21	—	Similar to left gland
SCM 28	1	4	14	10	Reduced to a few cells among fibrous tissue
SCM 7	6	8	21	13	Reduced to a few cells among fibrous tissue (fig 16)
SCM 12	6	7	21	14	Reduced to a few cells among fibrous tissue
SCM 10	—	—	21	—	Similar to left gland

The results summarised in Table IV show that independently of pregnancy, degeneration of the X zone during its later stages may proceed sufficiently rapidly for an appreciable amount of change to take place in 3 weeks though this is not always the case Degeneration was further advanced in the left gland of SCM 10 than in any other, but the X zone showed no appreciable change 3 weeks later

A comparison of the right and left glands of SCM 18 indicates that the gland remains unaffected during the first 6 or 7 days of pregnancy SCM 25 a slightly later pregnancy than SCM 18 showed active reduction taking place in the X zone of the right gland The process has been completed in SCM 28 (10 days pregnant) and later pregnancies The increased vacuolisation in the X zone of SCM 24 contrasts with the inappreciable change over a longer period in the non pregnant SCM 19 (fig 14) in which the same type of histological degeneration had reached a more advanced stage in the left gland Degeneration of the X zone thus takes place between the seventh and twelfth days of pregnancy, and coincides with the beginning of rapid growth in the embryos

Histology of Degeneration during Pregnancy —Degeneration is more rapid in the pregnant animal but does not differ in other respects from the process already described in the unmated female The degenerating cells of the X zone are in intimate contact with the medullary blood vessels, which are filled with red corpuscles

As long as it is intact the X zone appears practically lipoid free; during degeneration patches of lipoids occur in it, and also large vacuoles, of which some, but not all, contain fat at the time of fixation. After the X zone has undergone extreme reduction, the fibrous residue continues to separate the lipoid containing area of the cortex from the medulla; lipoids may extend up to this fibrous zone, but are commonly most plentiful near the exterior.

V.—*The Effect of Gonadectomy on the Adrenals.*

Castration.—14 male mice in all were castrated, 2 at 3 weeks old, 5 at 5 weeks old, and the remainder at 12 weeks or older. They were killed at intervals of 2 to 10 weeks after the operation; both adrenals were fixed and sectioned, and compared with those of normal mice of a similar age.

It was found that the glands of castrated animals showed a gradual increase in size over those of the controls, particularly when the operation had been performed prior to sexual maturity. The glands, being required for histological purposes, were not weighed, and estimations of the increase could only be made from serial sections. The glands increased up to 40 per cent. in thickness and were larger in section than those of the average male mouse; the largest observed approximated in size to those of the female. Where castration takes place after sexual maturity the adrenals enlarge more slowly, and probably never reach these dimensions; 8 weeks after the operation the increase in thickness is approximately 25 per cent.

Histological examination of the adrenals shows that the medulla takes no appreciable part in the enlargement of the gland. Three weeks after castration the cells of the zona reticularis have become more numerous; as they continue to increase (fig. 10) an area develops which eventually resembles the X zone in the young female in all respects. Fig. 4 is a section through the gland of a 13-weeks mouse, castrated at 3 weeks old, and shows the maximum development of this zone which was observed. It is interesting to note the absence of the fibrous reticular band which normally encloses the medulla in a male of this age. Apparently if the testes are removed at 3 weeks old, the partial degeneration of the inner zone of the cortex accompanied by proliferation of reticular tissue does not take place; the darkly staining inner zone (fig. 1) continues to grow, and remains interlocked with the medullary cells as in the young female.

The developing X zone in the male does not appear to be formed by an inward movement or proliferation from cells of the two outer cortical zones; these cells show no changes. The new zone arises on the site of the small normal zona reticularis, and spreads slowly outwards. At first the new cells resemble the normal reticularis cells of the male; they are similar in size to those of the zona fasciculata, but distinguishable by their darker staining cytoplasm and freedom from fat. The growing X zone forms a well-marked fat and lipoid-free area round the medulla; cell division is accompanied by a decrease in the size of the cells. In animals castrated after sexual maturity growth in the adrenal cortex appears to cease before the condition characteristic of the young female is reached. No further growth was found between 5 and 8 weeks after the operation, though the X zone was then neither as large nor as distinct as the one developing after castration in younger males.

Probably owing to the slow rate of growth mitoses are not common in any of the material examined, but some can be found in the developing X zone; in the same region some of the gland cells have constricted nuclei, suggestive of amitosis or of nuclear activity; these cells show no signs of degeneration.

Cortical hypertrophy of the adrenal following castration has been previously observed by Altenburger (1), Masui and Tamura (20) and Miller (21) in the mouse.

Ovariotomy.—As reported by previous workers (20, 21), ovariotomy seems to be without effect on the adrenal. Five mature mice were ovariotomised and the adrenals examined 16 and 30 weeks after the operation. After 16 weeks partial degeneration of the X zone had taken place, and after 30 weeks degeneration was complete.

In five other females the ovaries were removed at 24 days old, subsequent examination of the glands at intervals of 2, 4 and 12 weeks later showed that they had developed normally, and contained the usual large X zone. It follows that the growth and differentiation of the female gland is not dependent on the presence of the ovary.

VI.—*Discussion of Histological Changes.*

The significance of the degeneration occurring in the adrenal cortex of the mouse is unknown, but there can be no doubt that it is an entirely normal process often accompanied by hyperæmia. The latter appearance in the adrenal is constantly referred to as an indication of a pathological condition, but it may merely be associated with a phase of the life-history, or with the normal functioning of the gland. The fact that healthy mice, kept as far as possible under uniform conditions, may show considerable variation in the structure of the cortex, needs to be remembered in connection with experimental work on the glands of these animals.

The theory has been advanced by a number of writers (10, 11) that cell division in the adrenal only takes place in the outer layers of the cortex; there is held to be a constant inward movement of the cells, which eventually degenerate in the zona reticularis. Although a large amount of material has been examined in the course of this work, no evidence has been obtained in support of the theory, and certain observations imply that it is incorrect. Growth in the cortex of the male after castration, which is similar to normal growth in the female, appears to take place in the area round the medulla; an outwardly spreading undegenerate zone arises, containing mitoses, differentiated by its cell inclusions from the zona fasciculata. A somewhat similar zone may also develop in the female, after the original X zone has disappeared; this is transitory, but certainly not degenerate when first formed (fig. 17). Mitoses are rare in the adrenals of mice, except during their early development; figures suggestive of amitosis can be found, but these may merely indicate nuclear activity.

The Adrenals of other Mammals.—It is well known from the work of Armour and Elliott (2), Kern (16), Lewis and Pappenheimer (19) that a large part of the human adrenal degenerates shortly after birth. The developmental and post-natal changes have more recently been investigated by Keene and Hewer (15). Of the writers cited above, Kern gives the most detailed account of the histology of degeneration; except for the larger size of the degenerating cells in the human infant, the process there seems to be very similar to the degeneration in the cortex of the female mouse. It is interesting to note that, in both types of adrenal, degeneration is accompanied by the formation of a fibrous band round the medulla, at first fairly thick, but later becoming reduced and finally disappearing.

In connection with this point the glands of a number of other mammals were examined, and the following table summarises approximately observations on the presence or absence of fibrous tissue (F.T.) in the zona reticularis.

Table V

Animal	F T absent or scanty	F T diffuse	F T forms complete medullary band
Rat	3♀ 4♂	5♀ 4♂	
Rabbit	3♀ 5♂	8♀ 2♂ 2*	
Guinea pig	1♂	—	1♀
Cat	1*	1*	1♂
Dog	—	1♀ 1*	1♀ 1♂
Goat	1*	—	—
Ox or cow	—	1*	—
Primates			
Macacus	—	—	1*
Cercopithecus	—	—	1*
Cebus	—	1*	

* Particulars of the sex of the animals were lacking in some cases

It will be seen from the above table that considerable variation exists in the amount of fibrous reticular tissue present in the inner cortical zone of the mammalian adrenal There are indications that in mammals generally an increase in the fibrous tissue round the medulla is associated with a degeneration of cortical cells (*cf* Guieysse (8)) which takes place to an appreciable extent in the course of the normal life history

It has been shown (19) that the fibrous tissue formed during cortical degeneration in the human adrenal later undergoes partial or complete absorption so that its absence from an adult gland cannot by itself be considered an indication that degeneration has not occurred In some animals such as the rat where cell degeneration in the zona reticularis is believed to be gradual (12) diffuse fibrous tissue is not uncommon in that region in none of the rat glands examined however was there a well marked fibrous band round the medulla such as develops in the mouse after complete degeneration of the X zone This was also true of the rabbit adrenals which showed considerable variation among themselves as to the amount of fibrous tissue formed in the zona reticularis In the cat dog guinea pig and some Primates fibrous tissue may develop to a conspicuous extent and form a complete medullary sheath

Pregnancy and Cortical Degeneration—The adrenal cortex is commonly believed to undergo changes during pregnancy Watrin (26) assumes adrenal hypertrophy in the rabbit during pregnancy Kolmer (17) and Guieysse (8) have described histological changes during pregnancy in the guinea pig including vacuolation of cells and changes in the zona reticularis Donaldson (6) found no significant change in the weight of rat adrenals in the course of pregnancy. During the present work, a gland was examined from a rat killed immediately after parturition; there was a large intact reticularis zone which showed no changes comparable to those in the pregnant mouse.

This observation, together with those on the unmated female mouse, indicate that degenerative changes in the adrenal cortex are not specifically related to the pregnant condition, though they may be accelerated by the latter. Several male adrenals (other than those of mice) appear to show similar changes. It may be assumed provisionally that degeneration sets in at a certain stage of development, which varies somewhat in relation to the age of the animal, and may differ in the two sexes.

The appearance of the glands studied suggests that once the zona reticularis has degenerated in the young animal, the new inner zone, or adult zona reticularis, is commonly smaller and less distinct. It may also differ from the earlier one in its cell contents. It is hoped to make a further study of the appearance of the adrenal cortex at various ages and also during pregnancy in different mammals, as a preliminary to investigating experimentally induced changes in the gland.

VII.—*The Effect of Double Adrenalectomy on the Œstrous Cycle and Breeding.*

Most mammals fail to survive double adrenalectomy, but a large proportion of rats and mice appear to live indefinitely after the operation. It has been stated that this is because they possess accessory nodules of the cortical tissue, which is believed to be essential to life; these escape the operation, and may be present in sufficient quantity to compensate for the loss of the main glands. Jaffe (14) and Stewart (24) have recently reviewed the whole problem of survival after adrenalectomy, and their papers should be consulted for detailed information.

Adrenalectomised rats are said by Jaffe (13) to fall into three classes: (1) those dying soon after the operation, in which autopsy reveals little or no accessory cortex; (2) those dying some considerable time after the operation (up to five months), in which accessory tissue is absent or hypotrophic; and (3) those living indefinitely after the operation, which have relatively large amounts of healthy accessory cortex. The proportion of animals in each class may vary with the technique of the operation and the subsequent environment.

Stewart (24) considers that the whole question of accessory cortex needs further investigation. The present writer has found no satisfactory records of the presence of accessory cortex in mice; the latter show a gradation in the length of survival after double adrenalectomy similar to that observed in rats (4), but a higher percentage of mice survive indefinitely

Reproduction after adrenalectomy may be dependent on the functioning of accessory bodies where the latter are normally present and hypertrophic after the adrenals are removed they may be assumed to neutralise the physiological effects of the loss of the glands, in animals surviving sufficiently long Under these circumstances there is no reason why the ability of the animals to breed should be affected

Normal breeding after adrenalectomy has already been reported in rats (18) Rogoff and Stewart (23) found that pregnancy was not disturbed by adrenalectomy in dogs the survival period was actually lengthened Corey (3) however found that pregnancy and lactation did not affect the short survival period in adrenalectomised cats

The present data available for mice include (1) length of the oestrous cycle in the female (2) incidence of pregnancy size of litter etc (3) fertility of the male Data on the oestrous cycle in the rat after double adrenalectomy are given in Table VI

Table VI Length of Œstrous Cycle

	Rat	Mouse
Number of cycles observed before operation	2	35
Average length in days	84	5 12
Probable error	0 15	0 20
Number of cycles observed after operation	34	4 3
Average length in days	12	6 28
Probable error	0 34	0 19
Difference in cycle length	1 28	1 16
Probable error of difference	0 37	0 27
Difference probable error	3 40	4 26

Table V indicates that the oestrous cycle after adrenalectomy is about one day longer than before the operation the difference tending to be statistically significant It is hard to attach any definite biological meaning to this slightly longer cycle

Fertility of Females —Five litters have been produced from adrenalectomised females mated with normal males details are given in Table VII The average size of the litters is small but adrenal removal is clearly no bar to normal gestation

Table VII

No of female	Interval between parturition and removal of second gland	Size of litter	Sex of litter	
			Male	Female
	Days			
DBM 1	43	2	Still born	
DBM 3	67	1	Still born	
DBM 11	20	6	2	4
DBM 18	22	4	2	2
DBM 18	54	3	2	1

Fertility of Males —Double adrenalectomy was performed on 5 males of which SM 2 and 3 died 7 and 8 days after the operation and in consequence had little chance to breed The other 3 males proved fertile when mated with normal females Details in Table VIII

Table VIII

No of male	Interval between birth and removal of second gland	Size of litter	Sex of litter	
			Male	Female
	Days			
SM 1	25	3	0	3
SM 1	32	6	3	3
SM 4	31	6	Still born	
SM 4	26	2	1	1
SM 6	34	4	2	2

SM 1 4 and 6 were killed about 6 weeks after the second operation a post mortem examination was made in the hopes of finding accessory adrenal bodies Only one was found this was in SM 4 in the neighbourhood of the right kidney It was fixed in Ciaccio s bichromate formol acetic sectioned and stained with Ehrlich s hæmatoxylin and Sudan III Examination showed the presence of an irregular mass of chromophil tissue but no cortical tissue This accessory body had a diameter of 1 mm and was 0 6 mm thick when sectioned

Summary

(1) The histological difference between the adrenals of male and female mice reported by previous writers has been further investigated

(2) At three weeks old the adrenals are alike in the two sexes ; an inner dark staining cortical zone can be distinguished.

(3) Growth of this zone ceases in the male before five weeks old ; a small amount of degeneration takes place, and fibrous reticular tissue develops round the medulla.

(4) In the female this zone continues to grow until puberty ; it then occupies more than half the cortex. Later it degenerates slowly in the unmated animal, and normally disappears before the end of the reproductive period. The reduction of this zone is accompanied by a proliferation of fibrous tissue in the same region which persists after total degeneration has taken place.

(5) No correlation has been found between histological changes in the gland of the unmated mouse and the œstrous cycle.

(6) Complete degeneration of the inner zone of the cortex takes place between the seventh and twelfth days of pregnancy ; the histological changes occur more rapidly than those in the gland of the unmated female, but are otherwise identical with them.

(7) A new inner zone may arise later in the cortex , this, though similar to the earlier one is distinguishable from it, but is also of a transitory character.

(8) The effect of castration on the adrenal is to cause the growth of an inner cortical zone of the female type.

(9) Ovariotomy appears to have no effect on the adrenal.

(10) The histology of the adrenals in mice is discussed in relation to that of man and other mammals.

(11) Double adrenalectomy was performed on a number of male and female mice which bred normally after the operation. It was found that the œstrous cycle was slightly lengthened in unmated adrenalectomised females, but otherwise normal.

I wish to express my thanks to Prof. J. P. Hill, F.R.S., for his interest in this work, which was done in the Department of Anatomy at University College, London.

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DESCRIPTION OF PLATES.

Abbreviations.

b.v., blood vessel; *cap.*, capillary; *f.*, fibrous reticular tissue; *m.*, medullary cells; *vac.*, vacuoles; *z.g.*, cells of zona glomerulosa; *z f.*, cells of zona fasciculata; *z.r.*, cells of zona reticularis; *z.X.*, cells of X zone; *z.X.d.*, degenerated X zone; *z.x.*, inner cortical zone of young mouse.

The microphotographs are the work of Mr. F. J. Pittock. Figs. 1–7 inclusive show horizontal sections through mouse adrenals, and the remaining figures represent parts of similar sections at a higher magnification. Unless it is otherwise stated, the photographs are from sections approximately through the middle of the gland. The amounts of cortex shown in figs. 8–17, however, cannot be strictly compared, since the thickness of the cortex varies in different parts of the adrenal. All photographs except fig. 9 are from glands fixed in Bouin. A portion of the medulla is shown at the base of all high power photographs.

PLATE 11.

FIG. 1.—Section through adrenal of 19 days' male, showing dark inner cortical zone, interlocking with the medulla which is lighter staining. (Ehrlich's hæmatoxylin and Pasini. × 70.)

FIG. 2.—Section through a large adrenal from a 12 weeks' male, showing fibrous band round the medulla, and a small area adjacent to it, grading into the rest of the cortex, but more darkly stained. (Ehrlich's hæmatoxylin and Pasini. × 40.)

FIG. 3.—Section through adrenal of a 12 weeks' old unmated female, showing the large darkly stained X zone, and groups of cells belonging to it lying free in the medulla. Note the absence of fibrous tissue round the medulla. (Ehrlich's hæmatoxylin and Pasini. × 40.)

FIG. 4.—Section through adrenal of a 13 weeks' old castrated male, 10 weeks after operation. The gland is of the female type shown in fig. 3. (Iron hæmatoxylin and Van Gieson. × 40.)

PLATE 12.

FIG. 5.—Section through the gland of an unmated female after degeneration of the X zone; note fibrous band separating medulla from cortex. (Iron hæmatoxylin and Van Gieson. × 40.)

FIG. 6.—Section through right adrenal of female SCM 4, showing the zona reticularis which has arisen round the fibrous remains of the degenerated X zone. The section lies to the outside of the median plane of the gland. (Ehrlich's hæmatoxylin and Pasini. × 40.)

FIG. 7.—Section through the right gland of SCM 15, which shows some hypertrophy; the left gland was removed 7 weeks earlier. Description as that of fig. 6. (× 40.)

PLATE 13.

FIG. 8.—Section through adrenal of 33 days' male showing fibrous tissue developing round the medulla and degenerating cells in the medullary blood vessel. (Ehrlich's hæmatoxylin and Pasini. ×200.)

Fig 9 —Section through large adrenal of 9 weeks' male showing a well developed zona reticularis The section lies to the outside of the median plane of the gland, in order to show the reticulate arrangement of the cells next the medulla (Fixation in Flemming osmicated fats mostly dissolved by xylol, stained iron hæmatoxylin and Van Gieson × 200)

Fig 10 —Section through adrenal of adult male 5 weeks after castration showing zone of small darkly stained cells which is forming next the medulla (Iron hæmatoxylin and Van Gieson × 200)

PLATE 14

Fig 11 —Section through adrenal of unmated female OBM 2 showing large hyperæmic X zone (Ehrlich s hæmatoxylin and eosin × 200)

Fig 12 —Section through left gland of female SCM 7 removed before the animal was mated Note partial degeneration of the X zone indicated by the crowding of the outer cells and the development of fibrous reticular tissue The inner part of the X zone is still intact and shows a reticulate structure (Ehrlich s hæmatoxylin and Pasini × 200)

Fig 13 —Section through the adrenal of unmated female OBM 3 showing a later stage of degeneration of the X zone which has become smaller and less distinct Hyperæmia is apparent and large vacuoles can be seen next the medulla (Iron hæmatoxylin and Van Gieson × 200)

PLATE 15

Fig 14 —Section through the adrenal of unmated female SCM 19 showing extensive vacuolation of the X zone which has undergone little reduction in size, at the base of the figure darkly stained cortical cells can be seen projecting into the medulla. (Ehrlich s hæmatoxylin and Pasini × 200)

Fig 15 —Section through the adrenal of unmated female OBM 15 after degeneration of the X zone note development of fibrous reticular tissue round the medulla (Iron hæmatoxylin and Van Gieson × 200)

Fig 16 —Section through right adrenal of SCM 7 obtained when the mouse was 13 days pregnant note replacement of the X zone seen in fig 12 (SCM 7 left adrenal) by a small area containing fibrous reticular tissue blood corpuscles and degenerating cells (Ehrlich s hæmatoxylin and eosin × 200)

Fig 17 —Section through right adrenal of SCM 4 4 weeks after the removal of the left gland note development of the zona reticularis above the fibrous remains of the degenerated X zone This is the gland shown in fig 6 but under higher magnification. (Ehrlich s hæmatoxylin and eosin × 200)

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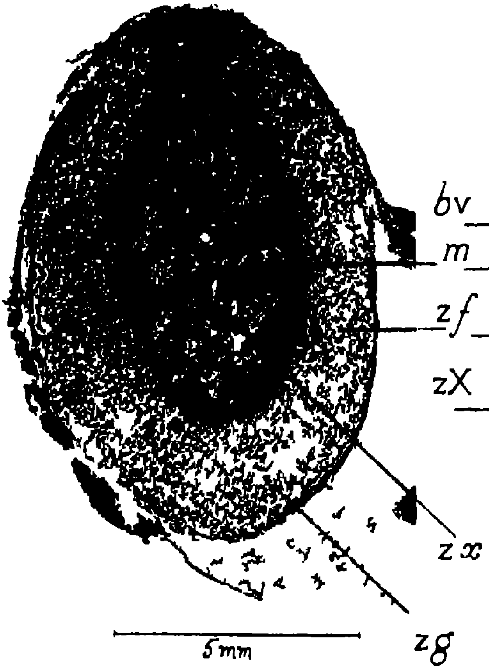

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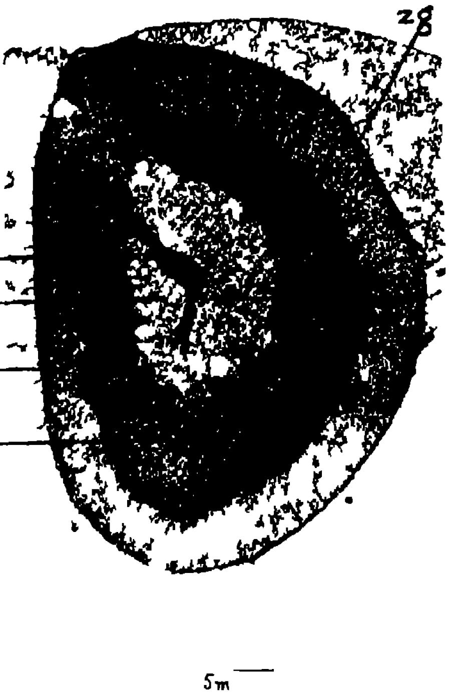

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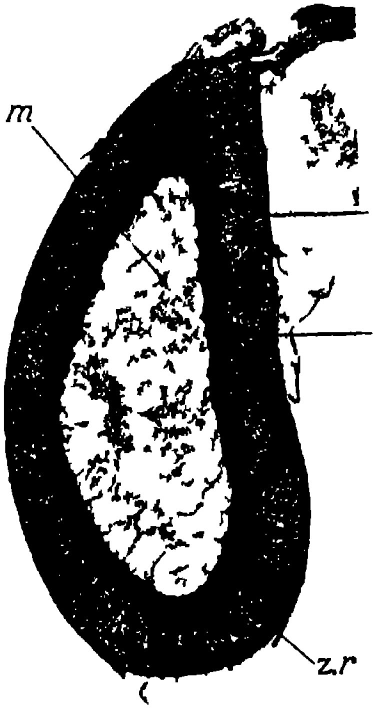

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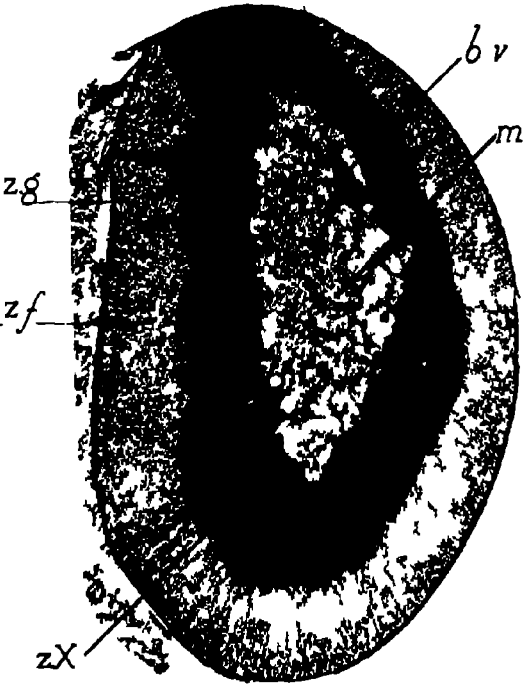

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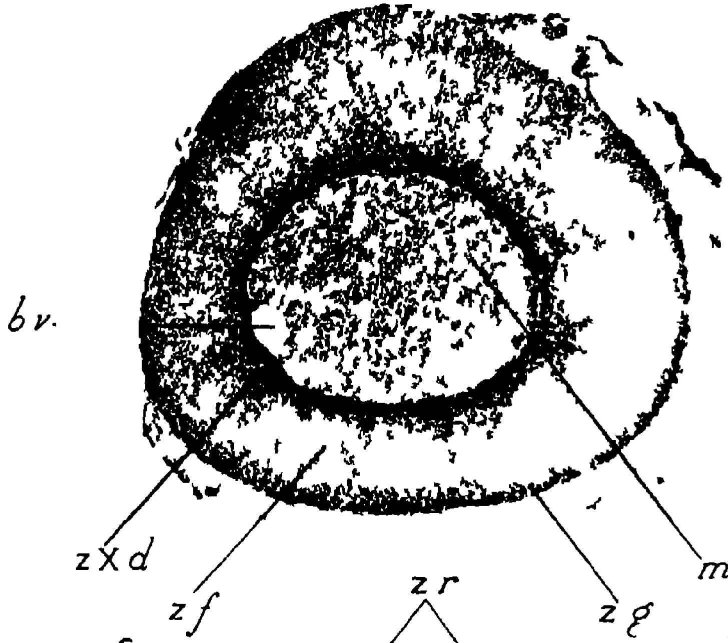

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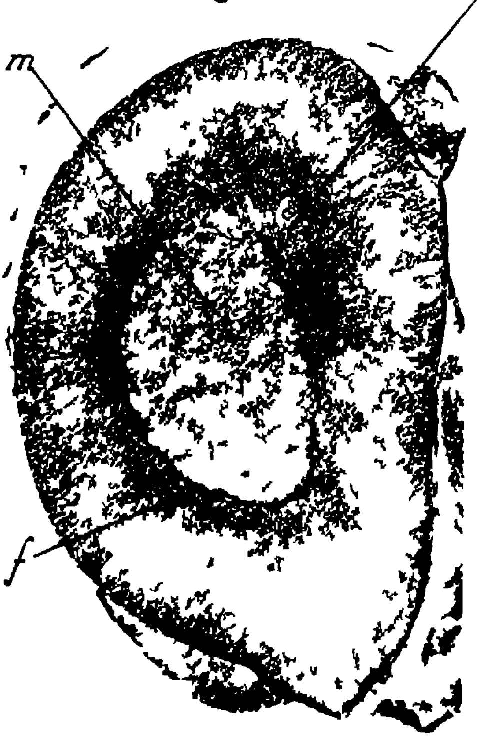

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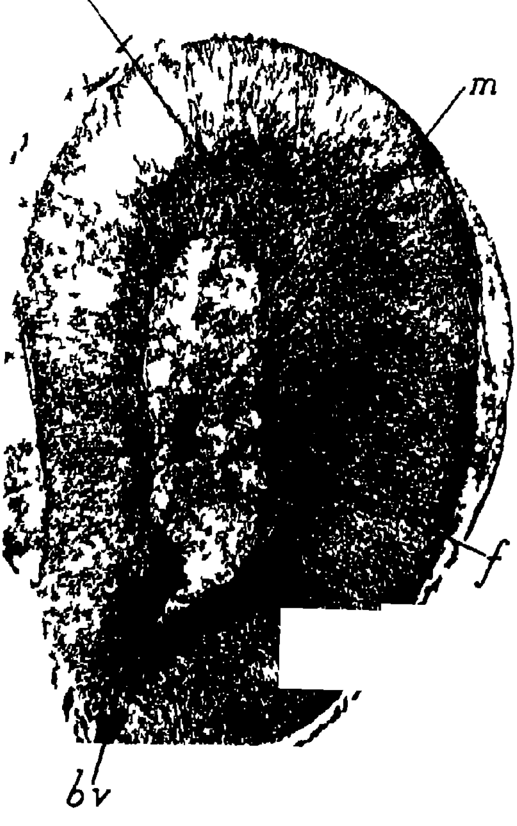

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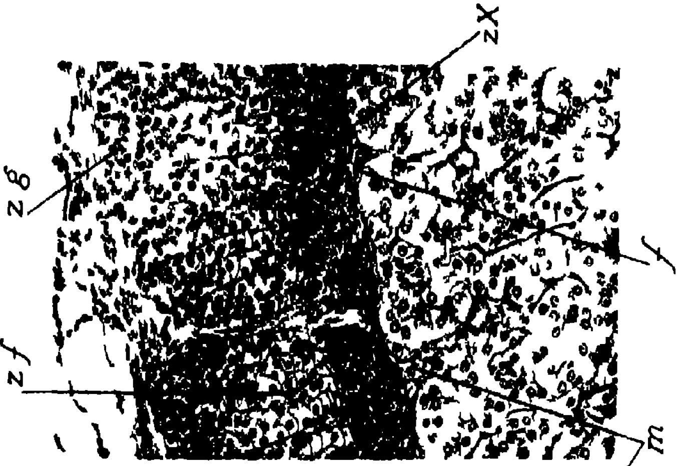

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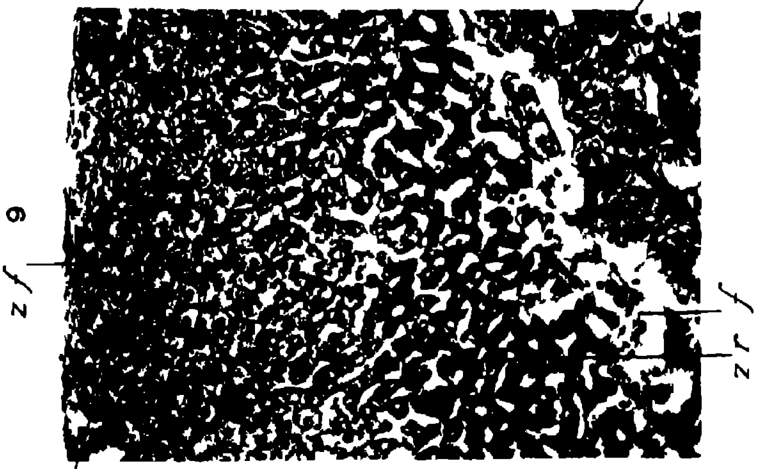

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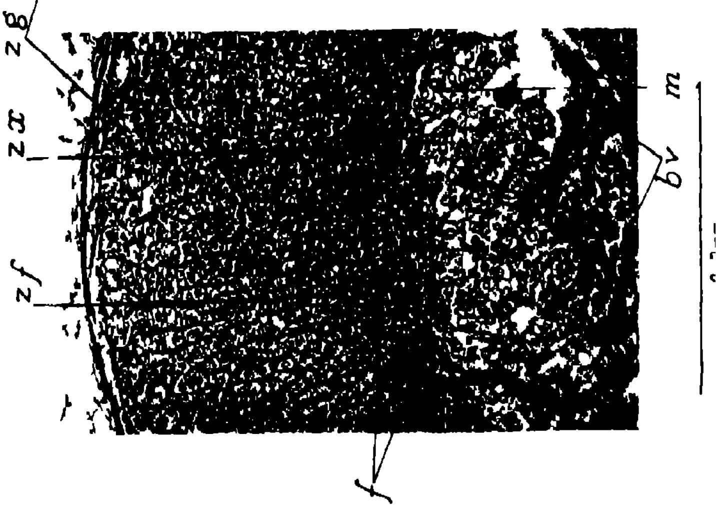

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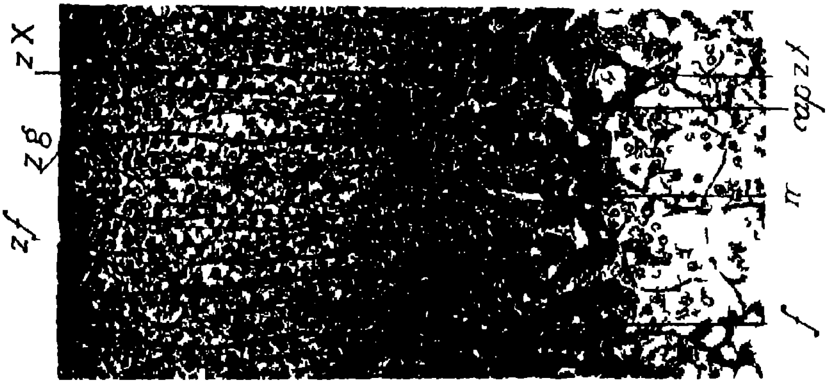

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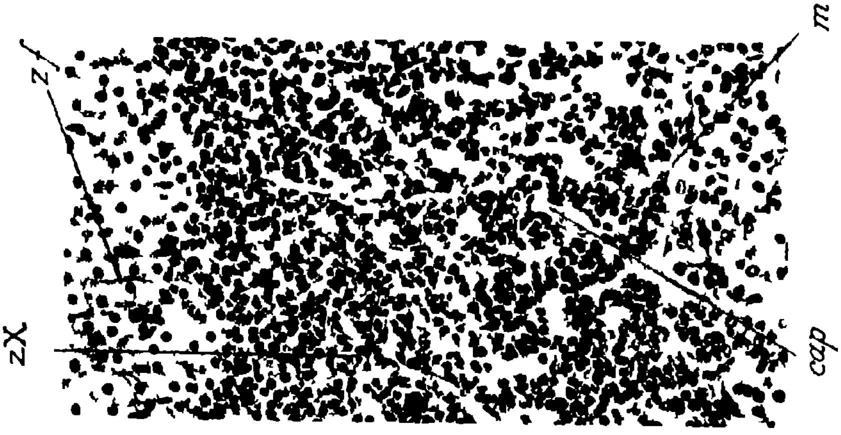

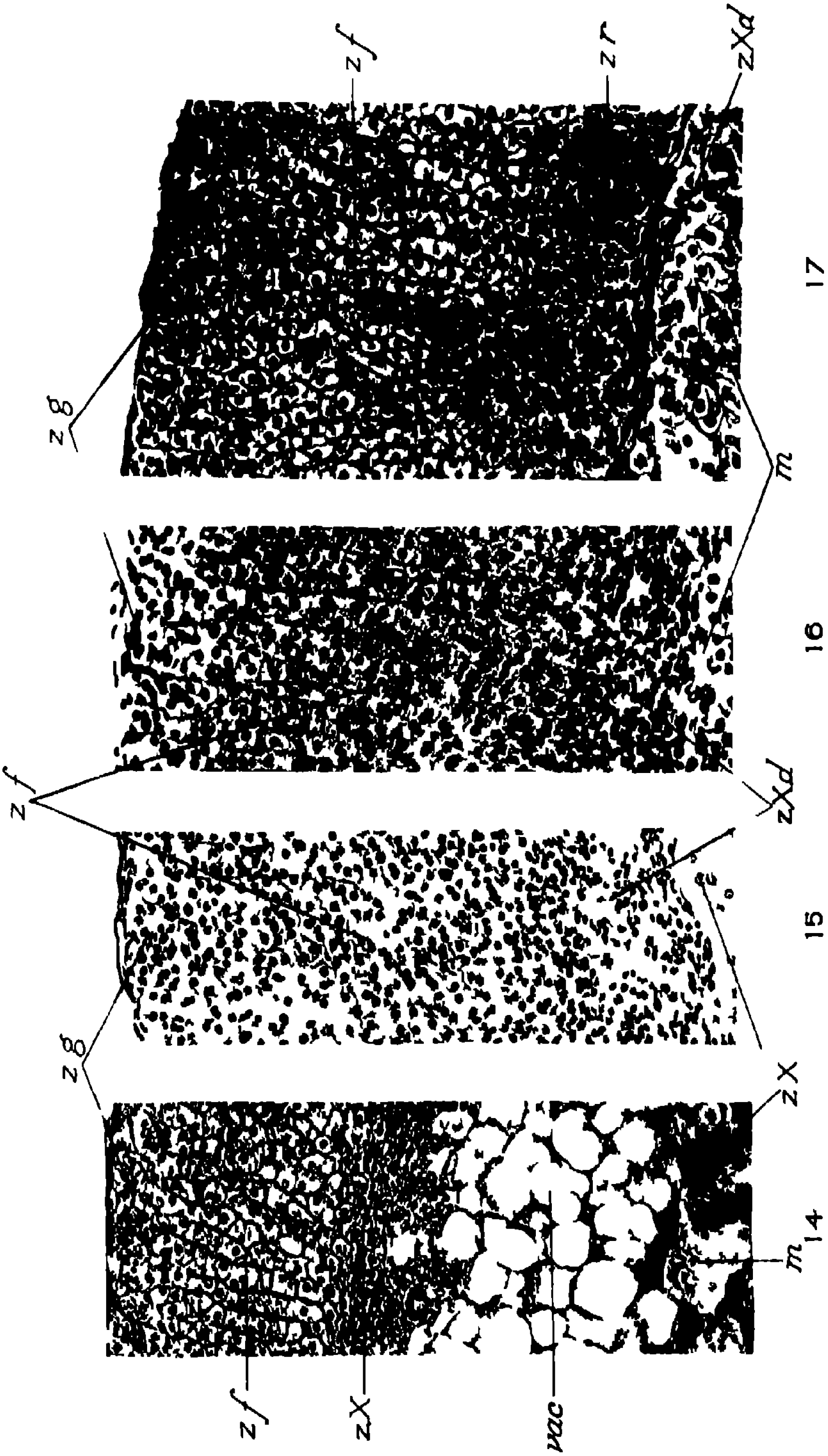
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Experiments on the Physiology and Genetics of the Smut Fungi Cultural Characters Part I —*Their Permanence and Segregation.*

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(Communicated by Prof V H Blackman, F R S —Received June 14, 1928)

[Plate 16]

1 *Introduction*

For some time the permanence of the characters of fungi when grown on artificial media has been the subject of research and considerable discussion has taken place on the origin of new forms (saltation) Many types have been studied but there has been no case as yet in which the characters of the parent organisms or of the saltants have been shown to be inherited in a fashion similar to those of the higher plants and animals

The investigation, the results of which are being published under the above general heading, was begun in 1925, and had as its objects (*a*) the solution of the question whether any cultural characters found were inherited along Mendelian lines, and (*b*) the breeding of certain strains for use in physiological experiments The organisms used were the Covered Smut of Oats (*Ustilago levis*, Kell et Sw) and the Covered Smut of Barley (*Ustilago hordei*, Pers) Certain cultural characters have been determined and experiments have been made which suggest that such characters are inherited in a Mendelian fashion The first part of this evidence will be given here namely evidence which suggests that the cytoplasm has no determining influence on these characters, evidence which shows that the characters of the strains have remained constant during the progress of the investigation, and, lastly evidence from which it may be deduced that the segregation of these characters may occur in either of the "reduction divisions."

Previous workers (1, 2, 3) have shown that the Chlamydospore (masses of which in the form of a dark brown powder replace the grain of the mature plant) is uninucleate, and that on germination a promycelium is formed into which the nucleus passes The reduction divisions of the nucleus follow, giving rise to four nuclei, which are distributed in a row along the promycelium Cross walls then divide the promycelium into four segments, and because no crossing over or passing of these nuclei has been observed, Kniep (4) has concluded that the nuclei are arranged in the segments in the order of their formation Each segment of the promycelium then proceeds to form sporidia or hyphæ according to the conditions each of which is uninucleate As described in a previous paper (5) the strains used in these experiments were obtained by the isolation and subsequent growth of such sporidia The next stage is the infection of the host plant and this is only achieved (6) by hyphæ which arise from the union of sporidia (or cells formed from them) of different gender (sex) Eventually the chlamydospores formed by the resultant bi nucleate mycelium replace the flower and grain of the host plant Thus it may be inferred that the cultures arising from the experimentally isolated sporidia are uninucleate and should be haploid

Both Reed (7) and Sampson (8) have shown the presence of physiologic races in the Covered and Loose Smuts of Oats by experiments on their capacity for infection While Christensen and Stakman (9) have described what may be cases of saltation in the Maize Smut but as the cultures did not arise from uninucleate cells they must remain under suspicion

Numerous cases of saltation have been described both in the fungi [*e g* Helminthosporium Stevens (10)] and in Bacteria [*e g* Dobell (11)] Brown (12) has shown that the tendency of certain strains of Fusarium to saltate is a function of the medium and found that it occurred frequently on a rich medium such as that of Richards and only rarely on a weak glucose asparagin mineral salts medium On the latter medium the individuality of all strains was easily preserved It must be remembered that he was dealing with a multi nucleate type whose complete life history (nuclear) is not known

Newton (13) has shown that in *Coprinus lagopus* sex is determined by two Mendelian factors which are carried by two different chromosomes and that the segregation of these sex factors may take place in either of the two divisions of the fusion nucleus

2 *Methods*

The methods employed were those usual in culture medium work For all experiments concerned with cultural characters the basic medium used was 1 gm KHPO ½ gm MgSO ½ gm KCl 1 5 gm agar and a litre of distilled water To this were added maltose and urea It was found that the most suitable ratio of the molecular concentrations of maltose and urea (symbol C/N) was 8 with a concentration of maltose of 0 0156 molar In all experiments to determine the constancy of the characters in the strains concerned, this ratio and concentration have been employed The *p*H of this medium (the agar being liquefied) was approximately *p*H 5 8 and the alteration due to sterilisation in an autoclave has never exceeded *p*H 0 2 That sterilisation does not affect this medium appreciably was shown by the test of comparison of growth on a batch of the medium made up by adding the other constituents, without sterilisation, to the previously sterilised agar, with growth on another batch on the medium made up in the usual way No difference of growth was discernible The total H ion concentration range explored was from *p*H 4 4 to *p*H 9 6 The determinations were made with a Hellige comparator, and the *p*H was always adjusted after sterilisation

As described in the previous article (5), the strains are for research purposes numbered according to the position on the promycelium of the segments from which they arise, the apical one being called /1, the penultimate one /2, and so on The four strains isolated from one promycelium are kept as one group, and numbered for example Z2/1, Z2/2, Z2/3, Z2/4 The stock cultures of these strains were kept on tube slants on a medium of 1 per cent Lemco beef extract agar, and subculturing was normally carried out once a week. In all petri dish experiments the parent cultures used were between a week and two weeks old

The temperature was that of the room experience having shown that within this range the variation of colony type was not significant Contamination was normally under 3 per cent No experiment was repeated less than three times and the number of replicate dishes varied from 4 to 16 in critical cases The methods used for isolation and cytological examination have already been described (5, 14)

3 *Cultural Characters*

As an example of the type of characters which these strains show in culture, reference may be made to Plate 16 which contains photographs of cultures from two sets of four strains, isolated previously described The parentage of the chlamydospores from which these strains were derived will be dealt with in the second part of this paper The characters which can be seen in such photographs are (1) the corrugation or depression at the centre of the colony, and (2) the amount of growth In the photographs in the Plate two of the strains in set Z/– show the character of corrugation and two show that of depression at the centre of the culture In the set 52/– two of the strains show a greater growth than the other two—the segregation is therefore, on a two and two basis This is not always the case, however, sometimes a 3 1 basis is obtained, and sometimes all four strains are the same, for example, see the character, centre of the colony in set Z/1 and Z/3 in Table III

4 *Permanence*

While it is now generally conceded that the seat of the genes determining the characters of an organism lies in the nucleus, yet in view of the perplexing nature of the occurrence of saltation in lower organisms it is thought advisable to give some evidence which strongly suggests that the cytoplasm has no determining influence on the characters dealt with in this paper

This evidence was the result of an observation described in a previous paper (5) Here it was shown that by appropriate treatment a "fusion" hyphæ is formed after the conjugation of two hyphæ of different gender It was shown further that in such hyphæ the nuclei lie side by side, and that the two cytoplasms were to all appearances completely mixed Further, that uninucleate conidia and hyphæ arose from these fusion hyphæ and that these offspring hyphæ had the same gender behaviour as the parent hyphæ From a number of fusion hyphæ formed from different parent hyphæ, the offspring hyphæ or conidia have been isolated and grown Parallel cultures have been set up of the parent strains and the offspring In all some 16 experiments have been set up involving some 800 cultures, and in no case in any of the characters described in this paper has any significant distinction been found between the parent strains and the offspring

While this offers no direct proof that the part of the cell concerned in the determination of these characters is the nucleus, it provides conclusive evidence that the influence of the cytoplasm on these particular characters is not of a determinate nature There is some slight evidence that, in some characters at least, the cytoplasm may have an influence, and until convincing proof of this or the contrary is obtained, such characters are not being used for any of the deductions from the experiments reported in this paper

As described in a previous section of this paper the strains used are uninucleate and are produced by the divisions following the reduction divisions Before describing any results on the segregation of the cultural characters in the reduction divisions it is necessary to show that segregation has not occurred in any other division of the nucleus under the conditions in which the stock cultures are kept, and the characters of the strains determined

Saltants are, as previously mentioned, a well known occurrence in the fungi, and if Christensen and Stakman's (9) results are correctly interpreted Smuts are no exception But as regards the strains employed in this work, while no change has as yet been observed under the given conditions, which can be definitely interpreted as saltation, an appearance similar to saltation has been obtained when a bi-nucleate cell was used as the origin of a culture, an appearance which was shown to be due to the separation of uninucleate colonies with the characters of the parent cells from the bi-nucleate parent (see reference in (5) to culture No. 39). While some work has been done and is being continued under conditions similar to those suggested by Brown's work on *Fusarium* (12), the results are as yet indeterminate.

That segregation does not occur under the conditions in which the stock cultures are kept, or under the conditions in which the cultural characters have been determined, is shown by the results of the following experiments. From the set of four strains 52/1–4 isolated in November, 1925, petri dish cultures were set up in the following January and examined after two months. The medium employed was 1·5 Lemco beef extract agar. The results showed that strains 52/1, 3 were hyphal and spreading in growth and 52/2, 4 were conidial and compact in growth. This experiment was repeated in January, 1928, and a similar result recorded. A repetition of the experiment illustrated in Plate 16 was made a year later and a similar result obtained. It must be noted that between these dates the stock cultures have repeatedly been started from single spore isolations.

To determine whether segregation under the conditions of germination was or was not complete in the promycelium, a number of the successive sporidia formed by each segment of the promycelium were isolated, and it was found that in every case the cultures arising from the sporidia formed by the same segment of any one promycelium were the same.

From these sets of experiments it is concluded that (1) under the conditions of germination, and (2) under the conditions in which the stock cultures are kept, and (3) under the conditions in which the characters are determined, segregation only occurs in the three nuclear divisions preceding wall formation in the promycelium.

That the strains used in this work are not artificial is shown by the fact that the experiments on infection described in a previous paper (6) were carried out with the strains 52/1–4, and that these were shown to be capable of causing a 91 per cent. infection.

5. *Segregation.*

It has already been mentioned that Kniep (4) after a study of the gender, behaviour, came to the conclusion that segregation occurs in the two parallel divisions in the promycelium. At the start of this work a number of experiments were made on the distribution of sex or gender in the promycelium. The results showed that Kniep's conclusion was only partially correct, but, taken by themselves, these results did not yield sufficiently clear conclusions to justify publication at that time.

The method of testing the gender reactions has already been described (5), and using this the reactions of some 90 sets of four have been analysed under certain known conditions of germination. It has previously been pointed out that in any set of four, two are always of one gender and two of another, and such being the case the possible distribution of these in the segments of the promycelium are as follows :—

Table I.

No. of segment.	Type No. 1.		Type No. 2.		Type No. 3.	
1	A	B	A	B	A	B
2	A	B	B	A	B	A
3	B	A	A	B	B	A
4	B	A	B	A	A	B

Since in no case has any observer shown that crossing or passing of the nuclei takes place in the promycelium, and because the ratio of the genders is 2/2, then if segregation took place in the first division the only possible arrangement would be type No. 1 and its reciprocal as in the table. If segregation took place in the two parallel divisions of the promycelium then the arrangements types Nos. 2, 3 and their reciprocals would be obtained. If there were any sex polarity in the nuclei, then only one of the reciprocal pairs would be obtained.

The results of some 22 sets of four are shown in Table II. The origin of the chlamydospores was the same host plant, and the germination conditions were also the same. One gender is called A, the other B, following Kniep's example (4). The criterion for A or B is strictly arbitrary. As a standard of reference the gender of culture CSOK 52/1 is being called A.

Table II.

Possible arrangement of genders on promycelium	AABB	BBAA	ABAB	BABA	ABBA	BAAB
No. of promycelia obtained	3	5	6	2	3	3

From this table it may be seen that all arrangements are found; in addition it can be stated that there is no sex polarity because the ratio of the occurrence of A in the terminal position to B in the terminal position is 12/10.

When a similar analysis of the cultural characters is made, it is again found that all arrangements occur (see Table III). Not only do all arrangements occur but they may all occur in the four strains from one promycelium, that is in the same set of four strains.

Table III.

Strain.	Gender	Colour.	Centre of colony.	Growth at *p*H 5·4	Growth at *p*H 8.
52/1	A	Cream	Depressed	2	2
52/2	B	Cream	Corrugated	1	1
52/3	A	Brown	Corrugated	1	2
52/4	B	Brown	Corrugated	2	1
Z/1	A	Cream	Depressed	2	2
Z/2	B	Brown	Corrugated	2	1
Z/3	B	Brown	Corrugated	2	1
Z/4	A	Cream	Depressed	2	2
Z1/1	A	Yellow	Depressed	2	2
Z1/2	B	Yellow	Depressed	2	2
Z1/3	B	Yellow	Depressed	2	1
Z1/4	A	Yellow	Depressed	2	1
Z2/1	A	Brown	Corrugated	2	1
Z2/2	A	Cream	Depressed	2	2
Z2/3	B	Cream	Corrugated	2	1
Z2/4	B	Brown	Depressed	2	2
Z3/1	A	Cream	Depressed	2	2
Z3/2	B	Brown	Depressed	2	2
Z3/3	A	Cream	Depressed	2	1
Z3/4	B	Brown	Corrugated	2	1

An example of this is seen in Z2/-. Here the gender distribution is type No. 1, the form of the centre of colony is distributed according to type No. 2, and the colour of colony distributed according to type No. 3. The segregation ratios of 3/1 and 4/0 are given to complete the table, and their presence is that which would be expected if these characters were inherited along Mendelian lines.

Although the presence of chromosomes has not yet been established in these organisms, the evidence would appear to justify the conclusion that the segregation of the cultural characters may take place in either of the two reduction divisions. It has not yet been determined that the segregation of any one character is not irrespective of what happens to any other character, but if this should occur it is probably a parallel behaviour to that of linkage as found in the higher plants and animals.

6 *Discussion.*

It would not be proper to discuss these experiments at this stage, for as already stated the concluding evidence that these cultural characters are inherited along Mendelian lines will be given in the second part of this article

In the introduction, Miss Newton's (13) conclusion that the segregation of the sex factors, carried in different chromosomes, took place in either of the two divisions of the fusion nucleus was mentioned In addition a similar observation has been made by Wenrich (15) who showed by cytological methods that in the spermatogenesis of *Phrynotettix* chromosome ' C " may divide reductionally in either of the two maturation divisions Thus it is seen that the deduction put forward here is paralleled by a similar behaviour both in organisms nearly related to the Smuts and in widely different organisms

The significance of this as yet apparently haphazard segregation seems likely from other experiments to be of considerable importance and it will be returned to in a later paper

The earlier experiments here described were carried out in the Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts with the aid of a grant from the Ministry of Agriculture and Fisheries, to whom thanks are due

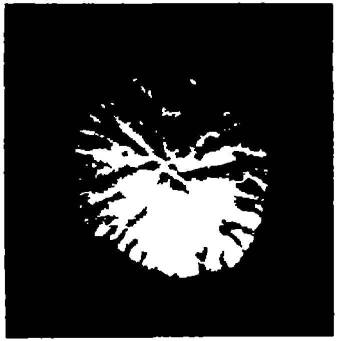
Summary

The Smuts Fungus used in the experiments described here is the Covered Smut of Oats (*Ustilago levis*) After isolating a chlamydospore and allowing it to germinate on a suitable medium, the first sporidium formed by each of the four segments of its promycelium was isolated transferred to test tube slopes and allowed to develop in culture Four cultures of strains were in this way obtained from one chlamydospore This has been repeated with a number of chlamydospores of known parentage

The strain obtained from any one of these isolated sporidia was found to differ in one or more cultural characters from the other three strains arising from the same chlamydospore A brief description of certain of these cultural characters is given

The segregation of these cultural characters was found to be on a 2 2, 3 1 and 4 0 basis It is deduced that this segregation may take place in either the first or the second of the ' reduction divisions " So far the segregation of any one character was found to be independent of that of any other

No conclusive evidence of somatic segregation has up to the present been obtained, the strains remaining constant during the time they have been in



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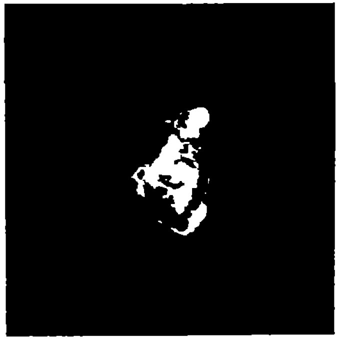

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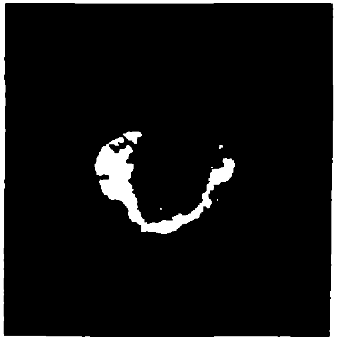

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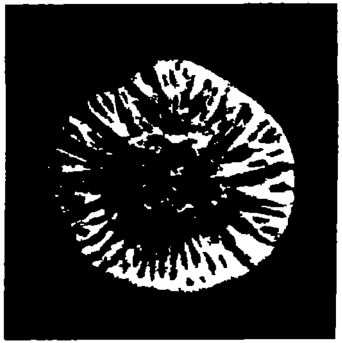

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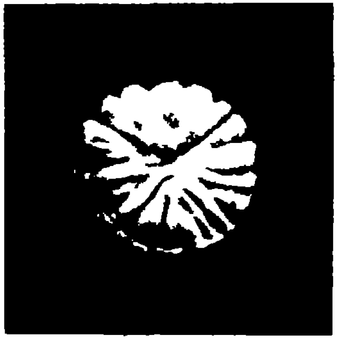
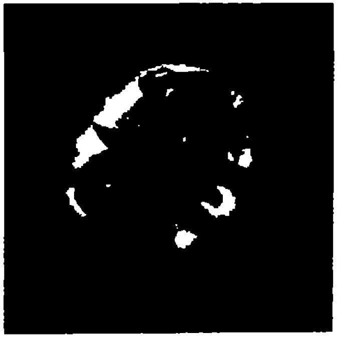
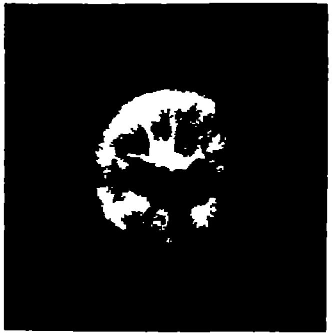


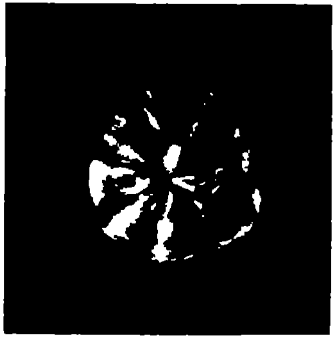
Z 1



Z 2



Z 3



Z 4

Photographs of Cultures of eight strains of *Ustilago levis*. The set of four strains S2 1–4 (figs 1–4) and the set Z 1–4 (figs 5–8) is each derived from a single chlamydospore as described in the body of the paper. The conditions of growth were the same in each case. Note in the set S2 1–4 the character amount of growth as shown by the diameter of the culture, and in the set Z 1–4 the character depression or corrugation of the centre of the culture.

culture. The cytoplasm has been shown to have no determining influence on the cultural characters so far described.

The concluding evidence that these characters are inherited in a Mendelian fashion will be given in the second part of this paper.

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Hæmolysis by Brilliant-Green and Serum

By Eric Ponder

(Communicated by Sir Edward Schafer, F R S —Received July 12, 1928)

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In earlier papers of this series (1, 2) I have described the rapid hæmolysis which results when small quantities of normal serum are added to systems containing sodium taurocholate or sodium glycocholate as lysins This obscure phenomenon depends entirely on the order in which the cells, the lysin and the serum are mixed If the lysin is added to the cells first and the serum added afterwards, the acceleration of hæmolysis occurs in suitable cases, whereas if the serum is mixed with the cells first and the lysin added afterwards, no acceleration but an inhibition of hæmolysis, is the result The effect of the addition of serum is similar for all types of mammalian red cells The component of the serum which is responsible for the acceleration appears to be a protein, for either serum albumin serum globulin, or hæmoglobin can bring about the effect, although egg albumin and gelatin are ineffective There is also some evidence that the acceleration on the addition of serum occurs with the lysins sodium oleate and potassium oleate, as well as with the bile salts (3)

In 1914 Browning and Mackie (4) described an action which occurs in hæmolytic systems containing red cells, serum, and brilliant green (tetra ethyl diamino triphenyl methane sulphate) The addition of small quantities of serum to systems containing red cells sensitised with the dye results in a very rapid hæmolysis, if however the serum is mixed either with the dye itself before the cells are added, or with the cells before the dye is added, inhibition of the slow hæmolysis which is produced by the brilliant green occurs The acceleration is therefore dependent on the order in which the components of the system are mixed A further investigation of the problem will be found in a paper by Mackie (5), in which it is shown that the serum component responsible for the acceleration is a protein, and that each of the protein fractions produces an effect Egg albumin and gelatin do not bring about the acceleration Dobner's violet, malachite green, ethyl violet, and methyl violet can be substituted for the brilliant green

As will be seen from these two summaries, there is at least a superficial resemblance between the acceleration produced by the addition of serum to systems containing sodium taurocholate and the acceleration met with in cell—brilliant-green—serum systems As Mackie points out there is perhaps a further and more important resemblance between these systems and complement amboceptor systems for in each case the cells require to be sensitised before being affected by the lysin This paper is concerned with the quantitative investigation of the acceleration phenomena in cell—brilliant green—serum systems and with an endeavour to find an explanation for the effect of the dye in producing a sensitisation of the cells

Material Used etc

1 *Brilliant Green* I have not found all specimens of this dye to be equally effective as a component of the hæmolytic systems Some specimens tested were found to be almost inactive little or no hæmolysis occurring when serum was added to the cells sensitised by them in the manner to be described below Other samples were extremely satisfactory but the reason for the difference I have been unable to discover In order that the results might be as comparable as possible with those of Browning and Mackie I have used in this investigation a specimen of brilliant green kindly supplied to me by Mackie himself and the same specimen as was used by him in his researches

The dye is made up in a 0 1 per cent stock solution in 0 85 per cent NaCl and kept in a Pyrex or Jena glass flask This is a point of importance since alkali from the glass quickly alters the stability of the solution and produces at the same time an opalescence This opalescence disappears on the addition of a few drops of dilute acid After some time the colour of this stock solution fades and the sensitising power becomes simultaneously reduced the solution should accordingly be not more than about a week old

2 *Cell Suspensions* —As in previous researches I use as an arbitrary standard a suspension of thrice washed cells from 1 c c of blood these cells being finally suspended in 20 c c of 0 85 per cent NaCl For a reason to be referred to below, it is essential that the washing of these cells be carried out very thoroughly, with large volumes of saline and that the cells be sensitised immediately after the preparation of the suspension

The sensitisation of the suspension is carried out in the following manner —To 5 c c of the suspension is added brilliant green, in quantities between 1 c c and 0 1 c c of the stock solution and the mixture allowed to stand for a length of time between 5 minutes and 1 hour at a temperature the same as that at which the subsequent experiments are to be performed The cells are then washed by repeated centrifuging until no dye remains in the supernatant fluid, and are then made up to the strength of the original suspension by the addition of 0·85 per cent. NaCl. The final suspension is very stable at ordinary temperatures, and shows neither the dusky colour which indicates the presence of brilliant-green nor spontaneous hæmolysis. Occasionally, and especially when high concentrations of the dye are used for sensitising (more than 1 c.c. of the stock solution to 5 c.c. of suspension), some lysis occurs during the washing; such suspensions must always be discarded. More rarely, the brilliant-green in the supernatant fluid shows an opalescence, and it is well to discard these suspensions also, at least in cases where quantitative experiments are to be carried out.

For a reason which will appear below, the cells must be sensitised at the same temperature at which the hæmolytic experiments are carried out. This is an unfortunate necessity, for it means that the centrifuge itself must be brought to the same temperature as the water-baths in which the experiments are performed, and that this temperature must be constant from day to day. I have been able to carry out these investigations in a room heated to 25°, but the necessity for controlling the temperature may easily give rise to difficulties.

3. *Sera.*—Except where otherwise noted, the sera used in this investigation are derived from the same animal from which the cell suspensions are prepared. In this way fallacies which might arise from the action of the serum of one animal on the cells of another are avoided. As a rule, the sera were separated aseptically and sealed into small sterile ampoules, a procedure which permits of the same serum being used several days in succession. Since serum which shows any marked degree of opalescence is useless for quantitative work, it is as well to obtain the serum immediately before a meal, or in the early morning. Plasma, obtained from oxalated blood, can be used in place of serum without any disadvantage.

The Nature of the Sensitisation with Brilliant-Green.

The results of the investigations into the physical nature of the sensitisation process which occurs when brilliant-green is added to the cell suspension is best expressed as a series of conclusions, accompanied by brief descriptions of method, etc.

(i) After the cells have been mixed with brilliant-green in suitable concentration and the dye removed as completely as possible by repeated washing, the cells will be found to have retained considerable quantities of the dye.

There is nothing in the appearance of the sensitised suspension to indicate the presence of this combined brilliant-green, for the sensitised suspension may have the same appearance as a suspension of normal cells ; large quantities of brilliant-green can be extracted, however, from the sensitised cells.

The extraction is best carried out with either alcohol or acetone, both of which are solvents for the dye. To one volume of the sensitised cell suspension are added two volumes of distilled water, and subsequently, when the cells are hæmolysed, five volumes of acetone or of absolute alcohol. The mixture is allowed to stand for some hours in stoppered vessels, and the concentration of dye in the clear fluid which overlies the hæmoglobin precipitate is estimated colorimetrically. The quantities of dye recovered in a typical experiment are shown in the following table, but it is to be noted that these quantities are rather variable, even under apparently the same conditions :—

Dye used for sensitisation.	Quantity recovered.
mgs	mgs
1·0	0·610
0·5	0·350
0·3	0·275
0·25	0·210

It will be observed that a proportionately greater quantity of dye is recovered from the cells when 0·25 mg. is used for sensitisation than when 1·0 mg. is used. I have several times attempted to show that the quantity of dye combined with the cells is related to the quantity left free in the fluid, by an expression of the type of the adsorption isotherm, but am not satisfied upon this point.

(ii) The union between the dye and the sensitised cells is a loose one, for such a simple procedure as warming the suspension is sufficient to make the cells liberate brilliant-green into the suspension medium. Suspensions which have been sensitised at 15° thus become dark after standing for a short time at 25°, and if such a suspension is centrifuged the dye can be seen to be present in what was previously colourless saline. Warming the suspension to 37° liberates large quantities of dye, and the warmed suspensions take on a distinctly dusky tint.

The fact that the union of the dye with the cells depends upon temperature in this manner makes it necessary that there shall be no temperature change between the time when the cells are sensitised by the addition of the brilliant-green and the time when the hæmolysis experiments are completed, for any alteration in temperature will result in an alteration in the quantity of dye bound to the cells The sensitisation the washings and the hæmolytic experiments must therefore be carried out at constant temperature, as has already been mentioned

(iii) The fact that the dye which is bound to the cells can be extracted with such solvents as alcohol and acetone and can be liberated by increasing the temperature suggests that the union between dye and cell is of the nature of an adsorption It is therefore not remarkable that the dye can be liberated from the sensitised cells by the addition of a surface active substance such as saponin The most instructive way of studying this liberation is to hæmolyse the sensitised cells with two volumes of water and then to add a small quantity of saponin after hæmolysis is complete The lysis by the water results in a red fluid which retains its colour indefinitely thus showing that the mere rupture of the cell is not sufficient to cause the combined dye to be liberated The subsequent addition of the saponin however results in a rapid darkening of the red fluid which after some hours may appear almost black If this dusky fluid is examined spectroscopically the characteristic band of brilliant green will be seen in the red Warming accelerates the liberation of the dye It is an interesting fact that the addition of sodium taurocholate does not cause this darkening to nearly the same extent as does the addition of saponin

(iv) Quantitative experiments show that the dye does not unite with the cell membranes only but also with the contained hæmogoblin This is readily demonstrated by hæmolysing the cells with distilled water centrifuging off the ghosts and then warming the hæmoglobin stained fluid adding saponin to it or extracting it with acetone In each case dye is recovered in large quantities from the hæmoglobin

(v) The dye which has been extracted either from a sensitised suspension or from the hæmoglobin of such a suspension can be recovered from the acetone solution redissolved in water recovered by evaporation redissolved in saline and used for the sensitisation of fresh cells So far as I have been able to discover it is as effective in producing sensitisation as is fresh dye from which it may be concluded that the essential properties of the brilliant green are not necessarily altered by its uniting with the cells of the sensitised suspension in the sense in which we have been considering the union

(vi) Knowing that the brilliant green is combined in a loose manner with the cell envelopes and with the contained hæmoglobin I have endeavoured to show by spectroscopic means that it is present in the sensitised cells and by using a microspectroscope with a special form of illumination have been able to photograph the spectrum produced by the single normal red cell and by the single sensitised red cell Both spectra show the bands of oxyhæmoglobin but the absorption band produced by brilliant green is absent altogether The presence of the dye in the sensitised cell is thus not demonstrable spectroscopically although as will be observed below the sensitised cells sometimes have a rather dusky appearance under the microscope The failure to demonstrate the dye spectroscopically is not a very significant fact but it indicates that the brilliant green is not free within the cell and is in keeping with the idea of its being adsorbed to the cell proteins

(vii) If the sensitised suspension is allowed to stand at a temperature of about 25° the quantity of dye which can be extracted from it will be found to become less as time goes on so that after from 12 to 18 hours scarcely any of it can be extracted with acetone or alcohol further neither warming nor the addition of saponin cause a darkening by liberating free dye At the same time, however there is no significant alteration in the effect of added serum upon the cells *i e* the disappearance of the brilliant green is not associated with a change in the sensitisation From this we may conclude that even in the freshly prepared suspension the sensitisation is not dependent solely on the presence of extractable brilliant green in the system but on some other condition hitherto unmentioned

(viii) This disappearance of the dye from the sensitised cell suspension is best studied by first considering the effect of the addition of serum or plasma to a dilute solution of brilliant green The addition of about 0 2 c c of serum to 5 c c of a 1 in 8000 solution of the dye is followed by an almost instant fading of colour this fading continuing more slowly until after some hours scarcely any green colour remains A similar effect although not so well marked is obtained if to the same concentration of dye is added about 1 c c of saline derived from a red cell suspension which has stood for some hours and it may also be observed that the fluid removed from the cells by washing during the sensitisation process undergoes a progressive diminution in colour as time elapses This fading of colour is of course more marked when large quantities of serum are added to the solution of the dye and is most strikingly observed when the concentration of the dye is small

(ix) There seems to be sufficient evidence for supposing that the fading of the colour is due to a reduction of the brilliant green to its leuco base for the addition of a mild oxidising agent such as H_2O_2 restores the green colour to the solutions from which it has been lost The means by which the reduction is brought about are obscure, but it may be suggested that it is accomplished through an interaction between the dye and either the amino or the sulphur groups of the cell or serum proteins.

(x) The fact that a cell suspension may show a high degree of sensitisation after all the brilliant-green contained in it has been converted into the leuco-base raises the question as to whether the leuco base can itself produce sensitisation In order to decide this question, I have prepared the leuco base from benzaldehyde and diethylaniline in the usual way, carefully purified it, and tested its effect on cell suspensions The substance is sufficiently soluble in saline to allow of its being added to cell suspensions in approximately the same concentration as that of the dye when used to produce sensitisation, and there is no doubt from these experiments that it is almost, if not quite, as effective as is the dye itself The reduction of the dye to the leuco base is, therefore, not an essential part of the sensitisation process, for the sensitisation occurs when the leuco base itself is added

Summarising these conclusions regarding the nature of the sensitisation process, it appears that the process is one in which either brilliant-green or its leuco-base is adsorbed or otherwise combined with the proteins of the cell suspension.

We may regard the essential process, however, as one in which the dye or its leuco base is combined with some component or components of the cell membrane in particular, rather than as one in which it is combined with the cell proteins in general, and thus the question arises as to whether the combination referred to is a simple adsorption or a combination of a more permanent and specific nature Taking a general view of the type of reaction which we are considering, it would appear that the latter is the more probable, and this idea is borne out by at least two considerations In the first place, it is to be borne in mind that every substance which is known to sensitise red cells to the subsequent addition of serum (sodium taurocholate, sodium glycocholate, the soaps, the dyes allied to brilliant-green, and their leuco-bases) is itself a hæmolysin if employed in sufficiently great concentration This hæmolytic action is not the result of a mere adsorption, but is probably due to the lysin forming with the proteins of the cell envelope a compound which is more or less stable, and it is probable that the preliminary sensitising effect is due to much the same cause as is the hæmolysis by the sensitising substance when present in great concentration In the second place, we have at least one piece of evidence to show that the sensitisation results in a definite change in the resistance of the cells, for such sensitised cells are about three times as susceptible to the lytic action of acid. It is unlikely that such a change could be brought about by the mere adsorption of the dye.

It should be observed, however, that sensitised cells are not changed in resistance to saponin, and, as Mackie observes, there is no change in their resistance to alkalies

The Kinetics of the Hæmolysis

While it is an easy matter to observe in a quantitative way most of the phenomena with which this paper is concerned it is by no means easy to obtain strictly quantitative results The principal difficulties are associated with the sensitisation process, and may be considered under three heads

(i) In certain cases the addition of brilliant green to the cell suspension does not result in a complete sensitisation, as is shown by the fact that the further addition of serum, even in large quantities, does not bring about more than a partial hæmolysis A suspension so sensitised is of course, impossible to work with in the usual way, for end points corresponding to complete hæmolysis are never arrived at This incomplete sensitisation seems to occur particularly when the cells of the rabbit are used, and depends not merely on the quantity of dye used for the production of sensitisation, but on some property of the cells themselves For this reason rabbit cells are to be avoided in quantitative experiments such as those to be described below Even suspensions of human cells, which usually sensitise excellently, will exhibit this incomplete sensitisation if not thoroughly washed, in this case, however, the difficulty probably arises from another cause, some of the dye used for sensitisation being adsorbed to traces of protein in the fluid suspending the cells

(ii) If a given quantity say 5 c c of standard suspension is sensitised with decreasing quantities, 1 c c, 0 9 c c, , 0 1 c c of brilliant green the sensitisation produced by the various quantities of dye will be found to vary considerably on different occasions On one occasion, for example, the greatest sensitisation may be produced with 0 5 c c of the dye, on another occasion, however, even under apparently the same conditions, the greatest sensitisation may be conferred by only half the quantity I have not been able to discover the cause of this irregularity, but, since I am satisfied that it is dependent neither upon temperature nor yet upon the length of time during which the brilliant green is allowed to act upon the cells, I am inclined to believe that it is to be explained only as the result of peculiarities of the cells themselves, *e g*, by variations in the extent to which the dye becomes united with the proteins of the cell It will be obvious that this uncertainty in reproducing any particular degree of sensitisation must constitute a very serious difficulty in quantitative work

(iii) If the suggestion which has been made regarding the sensitisation phenomenon is correct, the degree of sensitisation will depend on the quantity of dye united with the cell proteins as well as, possibly, on the nature of the union, and will therefore not necessarily have any simple relation to the quantity of dye added Experiment, in fact, shows that no simple relation exists, and accordingly, since we cannot measure by any independent method the extent to which the union between the dye and the cell proteins has occurred, we are faced with the probability that one of the most important variables in the experiments must be left undefined The extent to which this state of affairs must complicate the problem will be easily appreciated, for the case is somewhat analogous to one in which it is required to investigate the kinetics of saponin hæmolysis without measuring the quantities of saponin used

Bearing in mind these difficulties, we may proceed to the consideration of the kinetics of the hæmolytic reaction Since it is the addition of serum to the sensitised suspension which results in lysis, the first point to be investigated is the effect of varying the quantity of serum added, and to do this we proceed in the following way —

To a series of tubes, each containing 5 c c of the standard suspension, are added decreasing quantities of the stock solution of brilliant green, *e g*, 1 c c 0 9 c c, , 0 1 c c, and 0 05 c c The tubes are allowed to stand for 30 minutes at 25°, the cells are then washed thrice with 0 85 per cent NaCl, and the contents of each tube brought back to their original volume of 5 c c by the addition of saline In this way a series of suspensions composed of cells which have been treated with different quantities of brilliant green are prepared, and it is usually found that at least one of these suspensions is highly sensitised

At this stage it is convenient to carry out a rough determination of the amount of sensitisation of the various suspensions A series of tubes are prepared, each containing 0 5 c c of the serum to be used, together with 1·1 c c of saline To each of these is added 0 4 c c of the suspension to be tested, and the time required for complete hæmolysis noted The cells of one of the suspensions, usually that in which the cells have been sensitised with 0 5 c c of brilliant green, will be found to hæmolyse more rapidly than those of the other suspensions these are selected as the most highly sensitised For reasons of convenience the experiment is proceeded with only if these most highly sensitised cells completely hæmolyse within about 3 minutes at 25°, a longer time for complete lysis indicating a rather poor sensitisation

The following table shows the typical relations between the time required for the complete hæmolysis of 0 4 c c of such a highly sensitised suspension and the quantity of serum added to bring about the lysis In all cases the quantities are so arranged that the total volume of the system is 2 c c In the case of this particular suspension sensitisation was brought about by the addition of 0 2 c c of 0 1 per cent brilliant green to 5 c c of standard suspension

Serum c c	*t* mins	Serum c c	*t* mins
0 5	3 2	0 025	4 5
0 01	3 25	0 020	5 25
0 05	3 50	0 018	6 25
0 0	4 0	0 0125	10 0

This table brings out two important points —

(i) Even by the addition of very large quantities of serum the time required for complete hæmolysis is not reduced below a certain very definite value in this case 3 2 minutes The curve showing the relation of *t* to the quantity of serum added accordingly does not pass through the origin but cuts the abscissa at a point which we shall indicate by *t* So steeply indeed does the curve turn downwards towards this point that it is very difficult to be certain that the addition of say 0 5 c c of serum results in any more rapid hæmolysis than does the addition of a much smaller quantity say 0 1 c c

(ii) As the quantity of added serum diminishes the time taken for complete

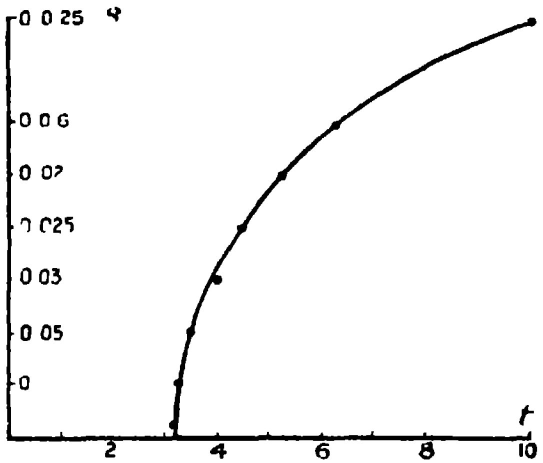

Fig 1

hæmolysis becomes longer and longer, until ultimately hæmolysis does not occur even after an exceedingly long time. This is best seen when the results are plotted, as in fig. 1, where the curve passes upwards towards an asymptote corresponding to the addition of about 0·01 c.c. of serum.

The next point for investigation is the effect of varying the quantity of brilliant-green with which the cells are sensitised. By adding increasing quantities of serum, we can obtain in each case a value t_m for the shortest time within which complete hæmolysis can occur, and can thus define one limit of the curve which shows the effect of various quantities of serum. The following table shows typical values of t_m, obtained with suspensions sensitised by adding decreasing amounts of 0·1 per cent. brilliant-green to 5 c.c. of standard suspension.

Dye added. c.c.	t_m.	Dye added. c.c.	t_m.
0·9	5·0	0·4	3·5
0·8	4·5	0·3	4·0
0·7	4·25	0·2	9·0
0·6	3·75	0·1	21·0
0·5	3·0		

The most striking point about these figures is one which has been already commented upon; the addition of a certain definite quantity of brilliant-green gives a maximal sensitisation and a minimum value of t_m, while the use of greater or of smaller quantities of dye for the production of the sensitisation results in this minimum time for complete hæmolysis being increased. In this particular case, the smallest value of t_m corresponds to the use of 0·5 c.c. of dye in the sensitisation process, but the quantity which results in maximum sensitisation is by no means a constant one. The maximum is, however, always clearly observable, and often even more marked than the above figures indicate.

In view of the manner in which we believe sensitisation to occur, it is not surprising that there is a particular quantity of dye which brings about a maximum effect, this quantity being that which unites with the whole of the cell component involved. Smaller quantities of dye we should expect to give a smaller degree of sensitisation, and thus the hypothesis accounts both for the appearance of a maximum value of t_m and for the value of t_m becoming greater as the quantity of dye used becomes less. At first sight it is difficult to see why the values of t_m should also become greater as the quantity of dye increases above that required to give maximum sensitisation; an explanation of this fact, however, is also indicated by the hypothesis, for under these circumstances there is excess dye in the system, and this excess dye is itself capable of reacting with added serum. The presence of the dye, in excess of that required to unite with all the cell component involved, may accordingly render ineffective a considerable quantity of the serum added. Unfortunately it is not possible to produce adequate evidence in support of this explanation, for the investigation of any form of inhibition in systems so complex as these is a matter of the greatest difficulty; I accordingly propose to omit all discussion of this possible inhibition in the meantime, and to confine myself to a consideration of the kinetics in systems in which there is maximum sensitisation, or sensitisation less than the maximum.

By adding various quantities of serum and observing the time taken for complete hæmolysis, it is possible to construct curves, such as that shown in fig. 1, for a number of suspensions sensitised with different quantities of brilliant-green, ranging from the quantity necessary for the production of maximum sensitisation to the quantity which produces so little sensitisation that experimental work becomes impossible. It should be observed, however, that such an experiment is very difficult to carry out in practice, for even if a number of suspensions are sensitised with different quantities of dye, it is unusual to find more than one or two in which the sensitisation is appreciably less than the maximum and yet is sufficiently great to allow of complete curves being obtained. The greatest number of curves which I have been able to obtain in any one experiment is four, and even this small number is not obtained in one experiment out of twenty.

The following table gives the essential data for a case in which four complete curves were investigated, the curves themselves being shown in fig. 2. In the case of the three curves last mentioned, a series of calculated values of t are given for comparison with the experimental values, the method of obtaining these calculated figures will be referred to below.

	Serum. c.c.	t, observed.	t, calculated.
5 c.c. suspension plus 0·3 c.c. dye	0·1	2·75	
	0·05	3·2	
	0·03	3·75	
	0·025	4·4	
	0·020	5·25	
	0·015	6·5	
	0·0125	9·0	
	0·010	15·0	

—	Serum, c c	t, observed	t, calculated
5 c c suspension plus 0 2 c c dye	0 1	3 75	3 70
	0 05	4 25	4 25
	0 03	5 0	5 0
	0 025	6 0	5 9
	0 020	7 0	7 0
	0 016	8 75	8 6
	0 0125	12 0	12 0
5 c c suspension plus 0 2 c c dye	0 1	4 5	4 25
	0 05	5 25	5 0
	0 03	6 0	5 8
	0 025	6 9	6 8
	0 020	8 0	8 1
	0 016	10 5	10 1
	0 0125	14 5	15 5
5 c c suspension plus 0 05 c c dye	0 1	18 0	17 6
	0 05	20 25	20 5
	0 03	24 0	24 0
	0 025	28 0	28 2
	0 020	34 0	33 5

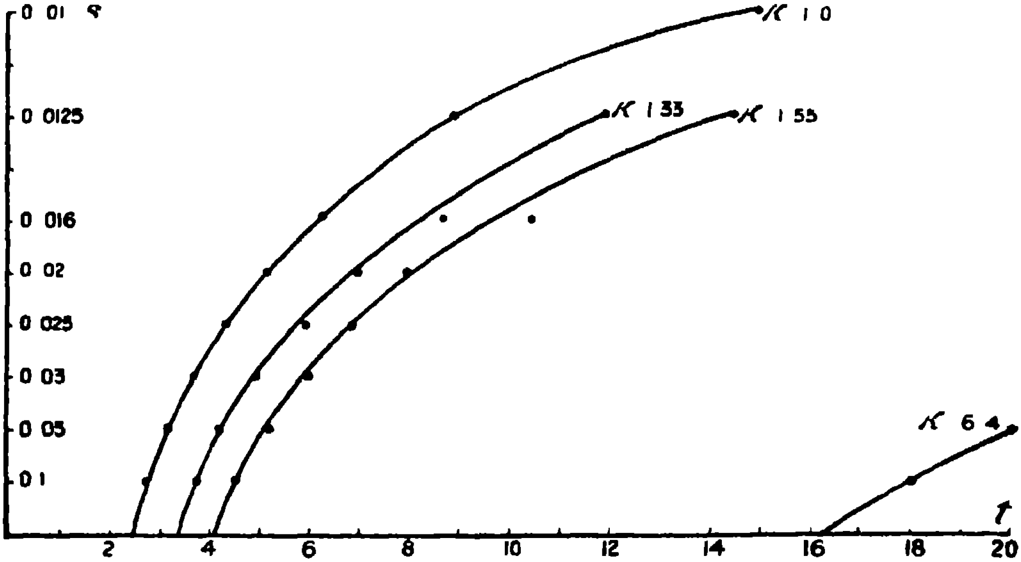

FIG 2

Two very significant points may be observed in connection with these four curves

(i) The four curves approach the same asymptote, for the addition of approximately 0 009 c c of serum gives hæmolysis in infinite time in each case From this fact we may deduce that the quantity of serum used up in the production of complete hæmolysis of the cells of the suspension is the same in every case, irrespective of the quantity of brilliant-green used for sensitising the system Further, since it is a matter of fundamental principle that the quantity of hæmolysin used up in hæmolysing a constant number of cells is itself constant, the fact that the quantity of serum used up is also constant suggests strongly that the serum added is either itself a hæmolysin or that it is involved in the formation of a new lysin within the system

(ii) The curves are related to one another in such a way that the time for complete hæmolysis in the case of any one curve differs from that in the case of another merely by a multiplying constant Thus if t_1, t_2, t_3, etc , are points on one curve, each point corresponding to the addition of a certain quantity of serum, and if t_1', t_2', t_3', etc , are points on another curve each corresponding to the addition of the same quantities of serum,

$$t_1' = k\ t_1, \qquad t_2' = k\ t_2, \qquad t_3' = k\ t_3, \quad \text{etc} \qquad (1)$$

It is from this relation that the calculated times in the above table are obtained, the curve for the suspension sensitised with 0 3 c c of dye being used as a standard since it provides the case in which the sensitisation is at a maximum, and the value of the constants being as follows —

Dye used for sensitisation	k
0 3	1 0
0 2	1 33
0 1	1 55
0 05	6 4

It will be seen from the correspondence of observed with calculated results that the relation expressed in (1) holds true with a remarkable degree of accuracy

The interpretation of the results of these experiments may be approached in two stages, the first being the explanation of the form of the curve as shown in fig 1, and the second that of the general relation expressed in (1) above As has already been observed, the evidence that a constant quantity of serum is used up in the hæmolysis of the sensitised suspensions can also be used as evidence either of the serum acting itself as a hæmolysin or of the serum being used up in the formation of a constant quantity of a new lysin within the hæmolytic system The former possibility, however, may be dismissed at once, for even the addition of the largest possible quantity of serum to the sensitised cells does not result in a reduction of the time for complete hæmolysis below the minimum time t_m, whereas, were the serum itself a hæmolysin, we should expect the curve under consideration to pass through the origin We have accordingly to consider the second possibility—that a new hæmolysin is produced within the system, and that this new lysin produces hæmolysis of the cells by acting as a simple hæmolysin

The simplest way of approaching the matter is to consider that the formation of the new lysin and its action on the cells of the suspension take place in two separate stages We shall suppose that a reaction occurs between the added serum S and a hypothetical component of the system A, the latter component appearing as a result of the sensitisation and that the result of this reaction is to form a quantity L of new lysin This quantity L will then be limited by two factors, the quantity of S present and the quantity of A present, L will in fact be proportional to the smaller of these two quantities just as in the case of a simple chemical reaction Under these circumstances, the velocity of the formation of the new hæmolysin will be

$$dL/dt = k_1 (A - L)(S - L) \tag{2}$$

and the time taken for the production of a constant quantity of L will be

$$t = \frac{1}{k_1} \log \frac{S(A - L)}{A(S - L)} \tag{3}$$

If the concentration of S is very great the quantity L of the new lysin will be formed exceedingly rapidly but at the same time the quantity formed will be limited by the fact that the quantity of A in the system is limited If on the other hand the concentration of S is reduced a point will be reached at which the amount of L is formed only after infinite time The curve for the production of a constant quantity of L limited by the quantity of A present in the system accordingly passes through the origin and proceeds to an asymptote corresponding to some concentration of S This curve is shown in fig 3 as the dotted curve 1*a*

The result of this reaction is the formation of the new lysin in quantity L and this new lysin now acts upon the cells of the suspension as a simple hæmolysin transforming a quantity x of some component C of the membrane and thus producing lysis The velocity of this reaction is

$$dx/dt = k_2 (C - x)(L - x) \tag{4}$$

and the time taken to complete the hæmolysis of the cells *i e*, for the transformation of a constant quantity of x, is

$$t = \frac{1}{k_3} \log \frac{L(C - x)}{C(L - x)} \tag{5}$$

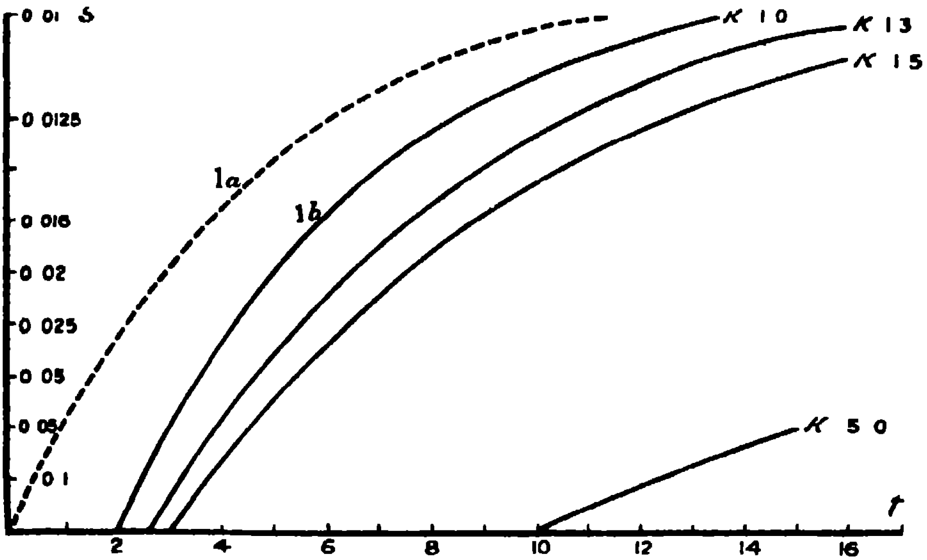

Fig 3

or, approximately if C is constant for the system and we are considering the formation of a constant quantity of x i e the lysis of a definite number of cells,

$$t = \frac{1}{k_1} \log \frac{L}{L - x} \tag{6}$$

As we have already seen, if A is limited in any particular system and S is added in very large amount the quantity of new lysin L will be formed in an exceedingly short time This quantity must however be limited by the quantity of A present and accordingly will take a certain time to complete hæmolysis, the time taken being given by expression (5) or (6) after the proper value of L is inserted In order to obtain the most rapid hæmolysis possible we require to add to the system a very large quantity of S in order that the quantity L may be formed instantly, but even under these circumstances we cannot obtain instantaneous hæmolysis, for the quantity of lysin formed, being limited, will still require a certain time to produce its effect, and thus the most rapid lysis which can occur in the system will be that which takes time t_m, which may be, for example, several minutes If smaller quantities of S are added, the same quantity of new lysin will be formed and will take the same time to produce hæmolysis, but its formation will be slower, as expressed in (3), thus the time which elapses between the addition of the serum and the completion of hæmolysis will be greater than t_m, and greater in proportion as the quantity of added serum is reduced Finally, when the amount of serum added reaches a limiting value, L will be formed only after infinite time, and under such circumstances the time taken for the hæmolysis will obviously be infinite also

The curve for the formation of the new lysin thus passes through the origin and approaches an asymptote corresponding to a certain amount of serum added (fig 3, curve 1*a*) The curve for the completion of the lysis, on the other hand, cannot pass through the origin (unless the concentration of A present in the system is infinitely great), but cuts the t-axis at a point t_m, the position of which depends on the value of A (fig 3, curve 1*b*) Both curves, however, approach the same asymptote, corresponding to the addition of a certain quantity of serum to the system

We have now to consider the effect of varying the quantity of brilliant green in the sensitisation process It has already been suggested that in this process the dye, or its leuco base, becomes united with a certain protein component of the red cell membrane In the nomenclature of the above equations, the dye thus combined would be denoted by A, while the cell component involved would be denoted by C, thus the quantity of C present in the hæmolytic system is dependent on A, which again we may suppose to be dependent, although probably not directly, on the quantity of brilliant green used for the production of sensitisation We have already seen that this hypothesis accounts for a certain quantity of brilliant green producing maximum sensitisation, this particular quantity being that which is combined with the whole of the cell component involved, and that which accordingly produces maximum quantities of A and C in the sensitised system At the same time it accounts for the relation expressed in (1) between curves for hæmolytic systems sensitised with different quantities of dye, for both A and C vary proportionately when the amount of sensitising dye is varied and thus the velocity of the formation of the lysin L and of its compound x with the cell component, is altered by a constant only

In this consideration of the occurrences within the hæmolytic system we have assumed that the formation of the lysin and its action on the cells of the suspension take place in two distinct stages This is, of course, unlikely, for it is almost certain that as soon as the lysin L is formed it commences to act upon the cells In order to express this it is necessary to solve expressions (2) and (4) simultaneously This in itself constitutes no difficulty, but a very serious difficulty arises as soon as we attempt to express the fact that the quantity of new lysin formed is limited by the quantity of the components A and S in the system, for in (2) we have to deal with a differential equation in which the velocity of formation of L may be very great if S is very great but in which the additional condition must be expressed that the quantity of L formed is strictly limited by the value of A I have been unable to obtain any solution of this problem in a form which can be used in practice principally owing to the number of undetermined constants which it is necessary to introduce and am therefore unable to do more than to show in fig 3 a series of curves which would be found for suspensions sensitised with different quantities of dye if the reactions discussed above occurred in two stages and to compare this series in a general way with the series found in experiment (fig 2)

In making the comparison two points should be noticed (*a*) So far as the more outstanding properties of the two series of curves are concerned *e g*, in their cutting the *t* axis in their approaching the same asymptote and in their bearing to one another the relation expressed in (1) the two series are identical (*b*) the curves in fig 3 are curves of simple first order reactions but those observed in experiment and shown in fig 2 are not for if we attempt to describe them by expressions of the first order we find that the velocity constant must be continuously reduced as the concentration of added serum becomes less This difference however is just what we expect to find if the reactions occurring in the hæmolytic system are consecutive instead of occurring in two stages and thus the difference between the two series of curves is in itself in support of the hypothesis advanced

We may summarise our conception of the mechanism of hæmolysis in these systems in the following way In the sensitisation process a compound is formed between a protein component of the red cell membrane and either the brilliant green itself or its leuco base We have no clear evidence as to the nature of this compound On the subsequent addition of serum the serum proteins react with the combined dye in the system to form a hæmolysin which can react with the protein component referred to, and which thus brings about lysis It will be observed that this hypothesis contains two essentials the first being that the hæmolytic reaction is really composed of two consecutive reactions and the second being that the new lysin formed by the combination of the serum proteins and the dye acts upon the same cell component as that to which the dye is united The dye thus acts as an amboceptor in an unusually strict sense of the term for it brings together the cell component and the serum proteins by first uniting with the former and then uniting with the latter

Changes in Form during Hæmolysis

In view of the importance attached to the changes in form of red cells during hæmolysis by different lysins it is convenient to record here the changes which occur when sensitised cells are hæmolysed by the addition of serum

The cells of a sensitised suspension are invariably found to be in the spherical or Goughian form and to present the typical fine crenations on their surfaces At the same time they may present a rather dusky tint As soon as serum is added the spherical form is replaced by the usual discoidal form this being the typical effect of the addition of serum Some of the cells may show irregularities of outline and the mottling of the envelope described by Millar (6) may be observed by means of dark ground illumination with the Cassegrain condenser

During the initial stages of hæmolysis no important change of form occurs After some time however and usually very shortly before the cell is to hæmolyse the discoidal form is again replaced by the spherical form no fine crenations are now visible but the mottling of the surface is quite distinct The cell then loses its hæmoglobin but the loss is not such a rapid one as for example in the case of saponin hæmolysis After lysis the cell envelope is clearly visible as a ghost and these ghosts may persist for a long time as observed by Mackie This fact suggests that the action of the lysin on the protein components of the cell membrane is not such a general one as in the case of the action of saponin

The changes observed are thus very similar to those which occur during hæmolysis by saponin and other simple hæmolysins

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Myograms yielded by Faradic Stimulation of the Cerebellar Nuclei

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Introduction

The general characteristics of the muscular manifestations which ensue on faradic stimulation of the cerebellar nuclei were described in a previous paper (28) In the present investigation an analysis of the nuclear responses was effected by recording the changes in tone in individual muscles of the fore and hindlimbs Prior to our own work faradic stimulation had been applied to the cerebellar nuclei by Horsley and Clarke (17) they did not record the muscular changes graphically but only observed the movements of the limb these movements to judge from their somewhat casual report resembled those observed by ourselves The belief that the responses observed by Horsley and Clarke were evoked by actual stimulation of the nuclei was clearly implied in their article but in a paper by R H Clarke (6), published posthumously the opinion is expressed that the reactions supposedly of nuclear origin were of a fallacious character depending on current diffusion to Deiters nucleus and various motor centres

Speaking more particularly for our own work we must state positively that our reactions were evoked by stimulation of the intracerebellar nuclei themselves, and were not due in any sense to spread of current beyond the cerebellum Phenomena caused by spread of current are readily recognisable and were avoided by using currents of threshold value for the nuclei, the excitability of which was carefully preserved. Our conclusions are thus more nearly in agreement with those of Horsley and Clarke than with those recorded in the posthumous paper of Clarke

Of interest in relation to nuclear stimulation is the work of Cobb, Bailey and Holtz (9), who applied faradisation to the *brachium conjunctivum*, whilst recording the tonus changes in the *triceps* muscles of both sides, the effects observed were inhibition in the ipsilateral, and increased tonus in the contralateral, muscle, an essential condition was the retention of the red nuclei

In the present paper, as in those preceding it (25, 28), the conception is developed of the cerebellum as an apparatus exercising both an augmentor and an inhibitory influence over postural tone, this view as to the dual nature of cerebellar control receives its support from the effects, already reported, of faradic stimulation of the cerebellar nuclei There result from nuclear stimulation muscular responses of widespread character involving muscles of the limbs, trunk, eyes and tail, the movements of the limbs are largely flexor in kind and those of the body are similar, in so far as they have been observed The movements of the limbs, as judged merely by inspection, appear to be coordinated in nature, there being increase of tone in the flexors and diminution of tone in the extensors or antigravity muscles, that the tonus changes are in reality of this kind is proved by the myograms presently to be described, which show, in the antagonistic muscles, phenomena clearly reciprocal in nature

Not only is an inhibitory influence on the tone of the extensor or antigravity muscles exerted by the cerebellar nuclei but a similar influence is exerted by the cerebellar cortex particularly its rostral portion, faradisation of which yields inhibition of decerebrate rigidity (36 27) Since this cortical region is connected with the *nucleus fastigii* (7, 15), which itself evokes inhibition of the extensors (28) (together with contraction of the flexors) we may infer the identical nature of the fastigial and cortical reactions

Reasoning from the results of stimulation just mentioned we are led to regard the cerebellum as influencing postural tone, so as to augment the tone of the flexor muscles and at the same time inhibit the tone of the extensor muscles, which appear to belong largely to the "antigravity group" It will be apparent that this conception of cerebellar function is not opposed to the views of de Barenne (2) and Rademaker (32), who reject hypotonia as a symptom of cerebellar deficiency The operation of cerebellar ablation was performed by them under strict asepsis and with careful anatomical controls, hypotonia they were unable to detect, though hypertonia appeared promptly, being expressed particularly as opisthotonus, retraction of the head, and extensor rigidity of the forelimbs, these symptoms of Luciani's first stage have already been interpreted as ' release phenomena ' (37), the consequence of the liberation of various centres from inhibitory control of cerebellar cortex and nuclei (25) On the basis of our theory hypertonia, not hypotonia, of the antigravity muscles is to be expected, on the other hand, hypotonia might logically be looked for in the flexor muscle groups

Now the methods used by de Barenne and Rademaker to examine muscle tone, such as by application of weights to the back of the animal or by palpation of the limb, would tend to reveal the state of tone in the antigravity muscles, whilst perhaps leaving unsuspected any possible hypotonia in the flexor groups But as to the hypotonic state of these after cerebellar removal there can be little doubt considering the augmentor effects evokable in them by nuclear stimulation Clearly then the results of de Barenne and Rademaker on cerebellar removal constitute no difficulties for our theory of dual cerebellar control over postural tone

A matter still demanding explanation is the frequent occurrence of hypotonia as a symptom of disease or injury of the human cerebellum, it was invariably found, for instance by Gordon Holmes (16) in his investigation of cases of cerebellar injury resulting from wounds received during the War the muscles affected were extensors of wrist and extensors of knee precisely those showing augmented tone after experimental removal The solution of the difficulty is probably to be found in the character of the clinical lesion which, being less clear cut than the experimental excision evokes not merely phenomena of deficiency but of irritation as well these latter may depend on the stimulating effect of increased intracranial pressure on the medullary centres (11) or on oedema of residual parts of cerebellar cortex and nuclei conditions which might conceivably lead to the discharge of impulses inhibitory to the motoneurones of the antigravity muscles In ways such as these may be explained the apparent discrepancy between the clinical and experimental lesions

It seems unnecessary here to review the controversy regarding the nature of muscle tone, we may merely remark on the prevalence to day of the views of Sherrington (41 42 21 44) that tone is always postural in kind being aroused and regulated (22) reflexly by stretching of the proprioceptors of the muscles themselves engaged in the particular posture, on the efferent side the discharge takes place through the somatic motoneurones which subserve ordinary muscular movement (46, 8 47, 10) In Fulton's (11) conception of tonus there is a rotational activity of the muscle fibres, following the ' all-or-none" principle and evoked as a series of stretch reflexes Assuming then the close similarity between tonus and ordinary muscular contraction we seem justified in referring to the positive effects evokable from the cerebellar nuclei either as contractions or as augmentations of tone, expressions thus regarded as practically synonymous, as to the negative nuclear effects, their envisagement as inhibitions of pre existing tone offers the simplest mode of expression

Anatomical Considerations

Whilst morphologically equivalent to the cerebellar nuclei of man, those of the cat are of somewhat simpler character, the several nuclei being closely connected together, the consequence of their origin in a single mass of grey matter (48, 33 29) Viewed in horizontal section the nuclear aggregate of each cerebellar moiety is obviously divisible into a medial part, the *nucleus fastigii*, and a lateral part this having a trilobed form, with one lobe extending forwards, one backwards and a third outwards, the lateral part includes the *nuclei dentatus*, *emboliformis* and *globosus* Weidenreich (48), whose studies extended over the cerebellar nuclei in various mammals, adopted designations for the nuclei differing from the conventional terms of human anatomy, in spite of certain advantages of his terms, those of human anatomy have been more generally employed by writers when referring to the nuclei of the lower mammals, they are used, for example by Winkler and Potter (49) in their atlas of the cat's brain as also by Horsley and Clarke (7) in their studies on cortical connections with the nuclei In these circumstances we have preferred to retain the older terms and have based our designations on the charts in the atlas of Winkler and Potter, thus the lateral nucleus, with slightly lobulated margin, is referred to as the *n dentatus* that next to it medially being the *n emboliformis* then the *n globosus* and most medially of all the *n fastigii* It is unnecessary, in this place, to enter more fully into the morphology of the nuclei and their connections in view of the somewhat extensive review of the subject in our earlier paper (28)

Methods

Prominent reactions which may be evoked by nuclear stimulation are flexion of the ipsilateral fore- and hindlimbs, these reactions being succeeded by extensor rebounds in both limbs (28) Guided by these reactions, we proceeded to record the tonus changes in typical flexor and extensor muscles in the fore and hindlimb on the side of the stimulation (the animal's right) The muscles selected in the forelimb were *biceps brachii* and *caput laterale* of *triceps brachii*

The *biceps* acts as a flexor of the forearm its origin by a tendon from the bicipital tubercle of the scapula makes requisite the fixation of this bone for purposes of recording Of the *triceps* the scapular head being employed reflexly as a flexor of the shoulder joint (41) we chose the lateral head for recording as being a pure elbow extensor the tendon of which could be readily isolated, the remainder of the muscle was rendered ineffective by disarticulation performed at the elbow joint

In the hindlimb the muscles used were the *tibialis anterior* and the *gastrocnemius soleus* pair The *tibialis* is employed as a flexor muscle in the flexor reflex The *gastrocnemius* although because of its origins potentially a knee flexor when used reflexly acts only as an extensor of the foot (41) also the *soleus* extends the foot from which it follows that the traction exerted by the two muscles through the *tendo Achillis* may be considered as purely an extensor effect

Considering first the experiments with the forelimb muscles our procedure was to isolate the tendon of the *biceps* and that of the *caput laterale* of the *triceps* in a preliminary operation performed aseptically under anaesthesia the tendons of the muscles freed to a suitable length were transfixed and ligated with silk threads amputation of the forearm was then performed through the elbow joint flaps of skin being sutured so as to cover the lower end of the humerus The immobilisation of an extensive group of muscles acting on the forelimb was accomplished in this stage by division of the *accessorius* nerve at its place of entry into the cleidomastoid muscle at the same time the procedure lessened the possibility of the obscuring of the results of cerebellar stimulation through current spread to the nucleus and rootlets of the nerve mentioned Experience showed us however that this consideration was not important since with the use of weak currents the dangers of current diffusion are easily avoidable the excitability of the nuclei having been well maintained by careful methods of preparation section of this nerve was therefore omitted in the hindlimb experiments which were completed in one stage

The actual experiment on the forelimb was performed under anaesthesia a couple of days after the aseptic operation After securing a cannula in the trachea the brachial plexus was exposed by an incision through the axillary region The musculocutaneous and radial nerves were then cautiously isolated and the remaining nerves of the plexus severed the trapezius group having already been paralysed in the first operation the *biceps* and *caput laterale* of the *triceps* were left to act unimpeded in the process of recording The wound in the axilla was then closed by suturing the skin Both hindlimbs were paralysed by nerve section but the opposite forelimb was left intact, the movements of the latter, together with the bodily movements, served as useful confirmation regarding the accuracy of our localisations

The carotid arteries were then ligated and the animal was placed prone in the usual decerebration position, the head being secured in a Czermak head holder Trephine openings were made on both sides of the skull, the intervening bone was removed and the calvarium was opened extensively, decerebration was through a plane cutting the thalamus dorsally about 7 mm in front of the superior colliculi and ventrally about 4 mm in front of the chiasma, meantime pressure was maintained on the vertebral arteries after the method of Sherrington Through the choice of the level of decerebration the integrity of the *nuclei rubri* was assured, a condition essential for our cerebellar stimulations

At a suitable interval after decerebration the right half of the cerebellum was exposed and a horizontal cut was made with a fine, sharp scalpel, so as to lay bare the cerebellar nuclei of that side, the plane of this cut lay in front about 1 mm dorsal to the paraflocculus laterally at the extremity of the inter crural sulcus and behind about 1 mm ventral to the upper margin of the paramedian lobule the incision was carried medially as far as the midline and a second vertical cut was made to meet it, in this way a block of cerebellar tissue was removed thus leaving the dorsal surfaces of the cerebellar nuclei exposed The excision was attended by a considerable amount of bleeding and we encountered great difficulties in controlling it, particularly in our earlier experiments Matters of importance were found to be the choice of a small animal and the maintenance of pressure on the vertebrals during and after the ablation the application to the bleeding surface of small pledgets of absorbent cotton soaked in adrenaline solution was also of great value The bleeding having been checked as far as possible, the skin flaps were closed with clips and arrangements were made for the actual recording

The animal was transferred to a special experimental table and was suspended by the head holder and either by a cord tied through the skin just in front of the tail or by a cotton sling, supporting the abdomen, the cord or sling was fastened in a suitable way above the animal Fixation of the fore limb was by a clamp gripping the spine of the scapula and by a second clamp of form specially moulded to grasp the lower end of the humerus, both clamps were fastened to uprights secured to the top of the table The tendon of the *biceps* like that of the *caput laterale* of the *triceps*, was connected, by a thread passing beneath a small pulley, to a crank lever writing on the Brodie-Starling kymograph Each lever pulled against a light flexible coiled spring the mode of recording thus being mainly isotonic the magnification of each lever was 3 6 times

The experiments on the hindlimb were performed in one stage Both forelimbs and the left hindlimb were paralysed by nerve section the muscles of the right hindlimb with the exception of the *tibialis anterior* and *gastrocnemius soleus* were rendered ineffective by nerve section or tenotomy The *nervus tibialis* was isolated at the ankle for stimulation in evoking the ipsilateral flexor reflex for purposes of comparison with the nuclear responses The tendon of the *tibialis anterior* was isolated and after detachment from its insertion was transfixed and ligated by a silk thread the *tendo Achillis* was dealt with in similar fashion Decerebration and exposure of the cerebellar nuclei were carried out as described in the forelimb experiment for hindlimb recording it was found unnecessary to elevate the animal which was left prone on the table the head secured by the holder Fixation of the hind limb was by a wide jawed clamp grasping the lower end of the femur and by a second clamp holding the foot The tendon of the *tibialis* and the *tendo Achillis* were connected by threads with the crank levers before mentioned the thread from the *tendo Achillis* passing under a small pulley

Stimulation was by the unipolar method using the stigmatic electrode of Sherrington the indifferent electrode was a saddle shaped brass plate covering a saline soaked pad applied to the clipped skin of the lower lumbar region the brass electrode was stitched to the skin The localising currents were of minimal intensity and using these it was possible to show that the responsive areas were strictly limited in various directions in this way one could be sure that the responses were yielded by the nuclei themselves and were not caused fallaciously through current diffusion to other structures The current was supplied by a Cambridge inductorium operated by three storage cells an ammeter and rheostat being included in the primary circuit the interrupter vibrated approximately 25 times per second

After exposure of the nuclei by removal of cerebellar tissue down to the level mentioned the stigmatic electrode was applied to each nucleus and the muscular responses were recorded we were guided as to the positions of the nuclei by the study of Weigert sections through the same level as that used in our stimulations , the various nuclei may be successfully stimulated along a line running transversely outwards from the level of the *sulcus primarius* in the intact left (remaining) cerebellar lobe since the nuclear substance extends forwards and backwards from this line the area of stimulation was enlarged accordingly The points in the nuclear areas yielding the most powerful responses were marked by the insertion of small bristles, quite often the extreme limits of the responsive area were marked by the same means, in such cases subsequent microscopic examination showed that a particular nucleus had been delimited and had thus, unquestionably, been the place of origin of the recorded responses

On the completion of an experiment the brain, after fixation in formalin, was photographed from above and below so as to show the positions of the bristles as well as the level of decerebration outline drawings were also made as guides to the photographs The portion of cerebellum, containing sometimes one sometimes several, bristles, together with the underlying brain-stem, was after subjection to the usual treatment embedded in celloidin, serial sections were cut through the sites of insertion of the various bristles and were stained by the Weigert hæmatoxylin method Microphotographs of the sections showing in each instance a bristle over a particular nucleus were finally made

Results Myograms of Forelimb Muscles

Nucleus dentatus —Faradisation of this nucleus yields muscular responses less intense, it is true than those from the other nuclei, but, nevertheless, definite in character The myogram of the *biceps brachii* shows, during stimulation, a smart rise then a rounded " ascent plateau turn " (19), followed by a plateau gradually declining and showing slight undulations (fig 1), following stimulation there is a quick fall devoid of after discharge, the latter being a noteworthy deficiency

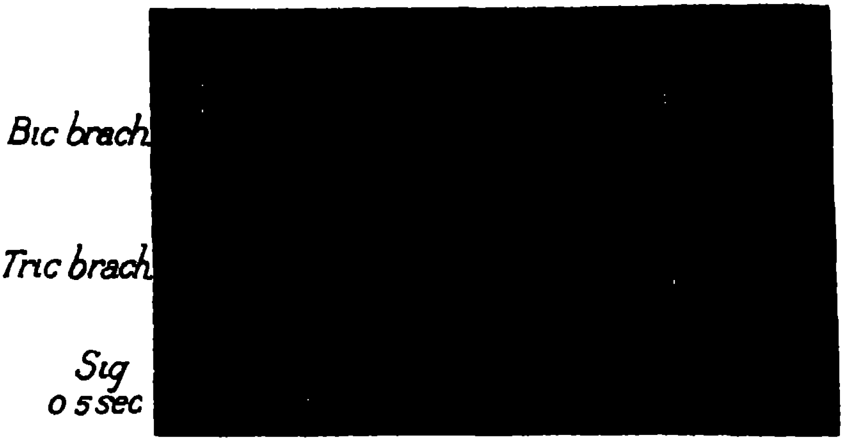

Fig 1 —Myograms of *biceps brachii* and *caput laterale of triceps brachii* obtained by unipolar faradisation of ipsilateral *nucleus dentatus* Sec dist 135 mm (see fig 1, Plate 17) *Note* —The myograms as reproduced are three quarter scale of originals.

The myogram of *triceps brachii* shows during stimulation a gentle slope downwards, after stimulation a slight rebound ' occurs The responses of the *triceps* to stimulation of this nucleus are not intense but when viewed in the light of the effects elicitable from the other nuclei it becomes clear that they are of the same kind, namely, that there is inhibitory relaxation during the stimulation, followed by rebound increase of tone thereafter, it is probable that the responses of *triceps* would have been more intense had its initial tonus been greater The behaviour of the two muscular antagonists both during and after the stimulation is obviously co ordinated exemplifying the principle of reciprocal innervation (39) The position of the electrode above the *n dentatus* of the animal's right side is shown in fig 1 Plate 17

Nucleus emboliformis—During the period of faradisation of this nucleus the *biceps* curve shows a smart rise with a rounded ascent plateau turn then a plateau which slopes downwards slowly, a quick drop without after discharge, follows the stimulation The *triceps* response consists in a gradual decline during the stimulus, to be succeeded thereafter by a definite rebound (fig 2) These phenomena, intrinsically like those on stimulation of the *n dentatus*, are here more strikingly portrayed, there is again adherence to the principle of reciprocal innervation, as before after discharge is lacking in the myogram of *biceps* whilst that of *triceps* shows rebound In fig 1 Plate 17 is shown the position of the electrode over the *n emboliformis*

Fig 2—Myograms obtained by unipolar faradisation of ipsilateral *n emboliformis* Sec dist 135 mm (see fig 1, Plate 17)

Nucleus globosus —The myograms obtained by stimulation of this nucleus are very like those from the *n emboliformis* there being the same reciprocal behaviour of both muscles here again after discharge is absent from the record of *biceps* As regards the *triceps* curve there is pronounced inhibition during the stimulus whilst post inhibitory tonus rebound is particularly striking (fig 3) If the initial *triceps* tone is marked its inhibition on stimulation is

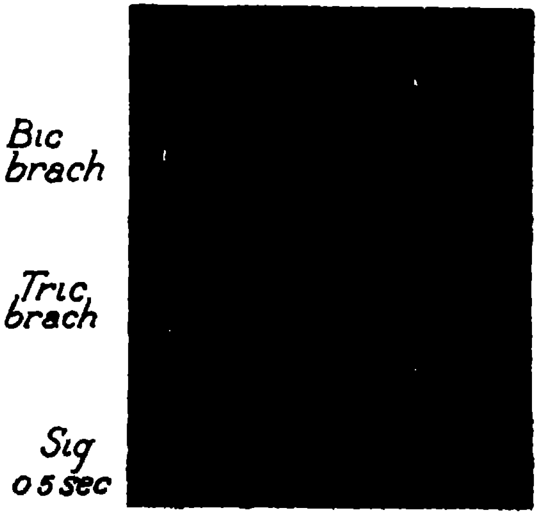

Fig 3 —Myograms obtained by unipolar faradisation of ipsilateral *n globosus* Marked inhibition of *triceps* tone followed by extensive rebound Sec dist 135 mm (see fig 1 Plate 17)

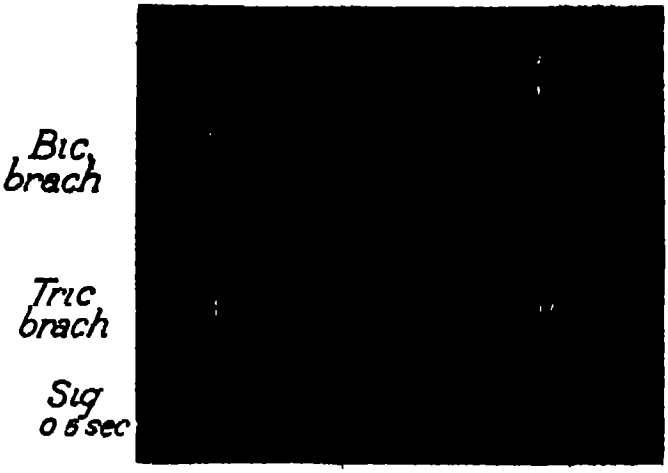

Fig 4 —Myograms obtained by unipolar faradisation of ipsilateral *n globosus* Slight inhibition of *triceps* tone followed by moderate rebound Sec dist 135 mm (see fig 1, Plate 17)

clear (fig 3), but if present in small amount muscular relaxation may not be observed, though the subsequent rebound shows clearly that the inhibitory influence had been at work on the centre (fig 4) A like independence of rebound of precurrent muscular relaxation is met with in the reflex (40) a circumstance suggesting the essentially similar nature of cerebellar and reflex inhibition and rebound The position of the electrode over the *n globosus* may be seen by reference to fig 1 Plate 17

Nucleus fastigii —The muscular responses evokable from this nucleus differ from those previously described merely in the exaggeration of their various phases The initial rise of the *biceps* myogram is smart and extensive the plateau flat or slightly falling (figs 5 and 6) The inhibition of *triceps* during

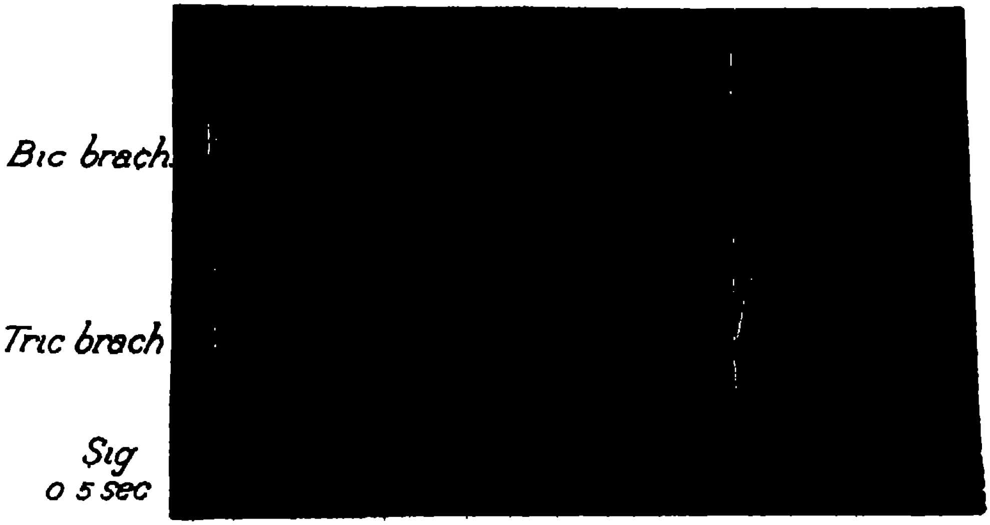

Fig 5 —Myograms obtained by unipolar faradisation of ipsilateral *n fastigii* Rebound after slight inhibition of *triceps* tone Sec dist 125 mm (see fig 1, Plate 17)

Fig 6 —Myograms obtained by unipolar faradisation of ipsilateral *n fastigii* Extensive inhibition of *triceps* tone

the stimulus is marked, given an initial tone of moderate intensity (fig 6), the post-inhibitory rebound of this muscle is powerful, rivalling or sometimes surpassing in height the excitatory contraction of *biceps*, once more rebound is observed, whether preceded or not by muscular relaxation during the stimulus (figs 5 and 6) The position of the electrode over the *n fastigii* for fig 5 is indicated in fig 1, Plate 17

Myograms of Hindlimb Muscles

Nucleus dentatus The hindlimb responses evokable from this nucleus, like those in the case of the forelimb, are weak in comparison with the responses of the more medially placed nuclei The myogram of the *tibialis anterior* exhibits, during the stimulus, a gradual rise in tone to a fairly flat plateau, at the close of the stimulus there is an immediate drop devoid of any after discharge The record of *gastrocnemius-soleus* shows a slight, gradual fall in tone during the stimulus and a slight rebound thereafter (fig 7) In fig 2 Plate 17, is shown the position of the electrode over the *n dentatus*

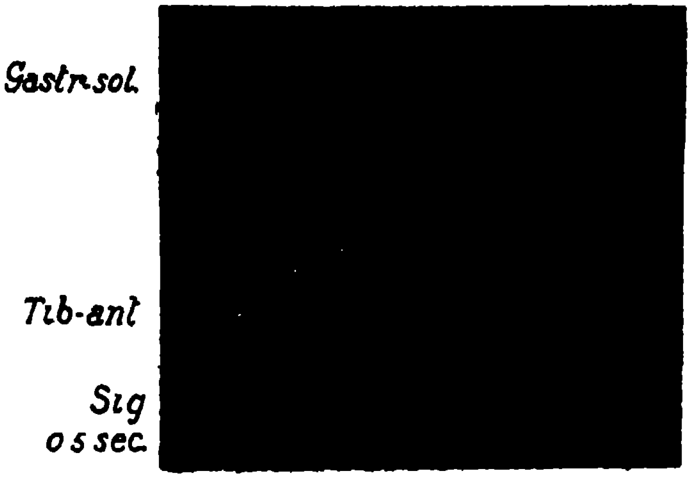

FIG 7 —Myograms of *gastrocnemius soleus* and *tibialis anterior* obtained by unipolar faradisation of ipsilateral *n dentatus* Sec dist 145 mm (see fig 2, Plate 17)

Nucleus emboliformis—The myogram of the *tibialis anterior* yielded by stimulation of this nucleus shows a prompt rise to a steady plateau, after stimulation there is a prompt drop in tone The myogram of *gastrocnemius soleus* shows a decline in tone during the stimulation followed by a slight rebound subsequently (fig 8) The position of the electrode over this nucleus is shown in fig 3, Plate 18

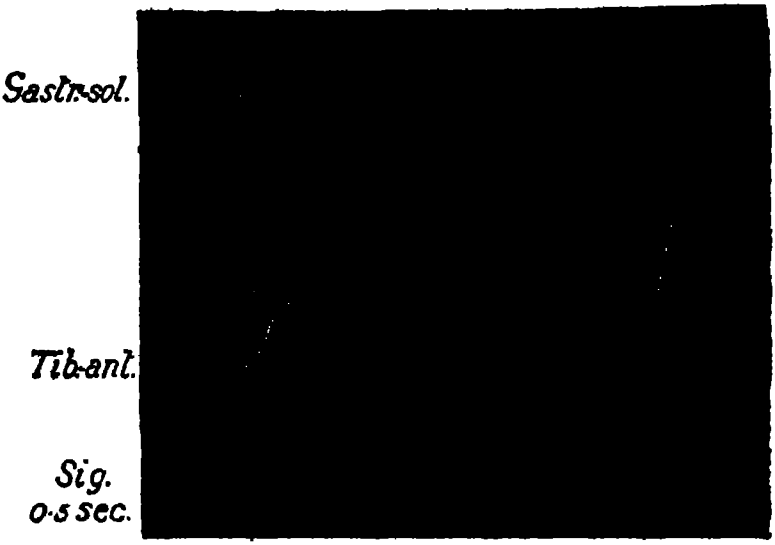

Fig. 8.—Myograms obtained by unipolar faradisation of ipsilateral *n. emboliformis*. Sec. dist. 145 mm. (see fig. 3, Plate 18).

Nucleus globosus.—The myogram of the *tibialis anterior* obtained by stimulation of the front part of this nucleus shows a large, sudden rise to a plateau,

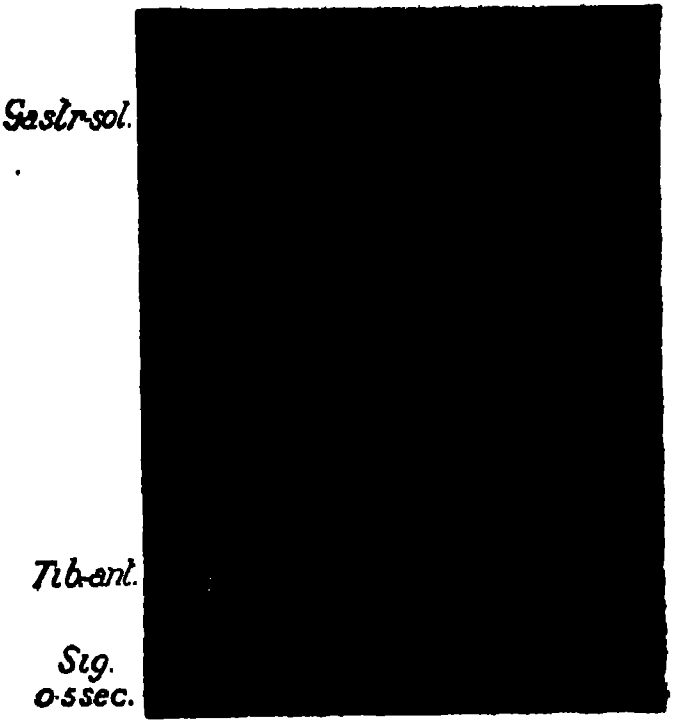

Fig. 9.—Myograms obtained by unipolar faradisation of front part of ipsilateral *n. globos*
Sec. dist. 145 mm. (see fig. 2, Plate 17).

which is briefly sustained; then follows a gradual fall. The *gastrocnemius-soleus* curve shows extensive inhibition during the stimulus, to be succeeded by powerful rebound on its cessation (fig. 9). The position of the electrode is indicated in fig. 2, Plate 17. A more steadily sustained plateau of *tibialis* contraction is yielded by the hind part of the nucleus (fig. 10), though here the *gastrocnemius-soleus* effects are slight. The position of the electrode in this latter stimulation is indicated in fig. 4, Plate 18.

FIG. 10.—Myograms obtained by unipolar faradisation of hind part of ipsilateral *n. globosus*. Sec. dist. 145 mm. (see fig. 4, Plate 18).

Nucleus fastigii.—The *tibialis anterior* curve exhibits, during stimulation of the forward-lying part of this nucleus, a quick, extensive rise, which is well sustained; following stimulation there is an immediate fall devoid of after-discharge. The *gastrocnemius-soleus* curve shows inhibition during, and rebound after, the stimulation (fig. 11). The relation of the electrode to this nucleus may be seen on referring to fig. 2, Plate 17. The hinder part of the *n. fastigii* yields reactions similar to those just described.

As a variant of these reactions, movements of progression are occasionally encountered; they are obviously reciprocal, activity of a protagonist co-existing with inhibition of its antagonist (fig. 12). These effects recall the progressive movements elicited from the front of the cerebellar cortex by Banting and one of us (F. R. M.) (27); doubtless, indeed, both reactions are basically the same, since the Purkinje cell axons in this cortical area are distributed to the *nuclei fastigii* (7, 15). The mechanism of progression set into

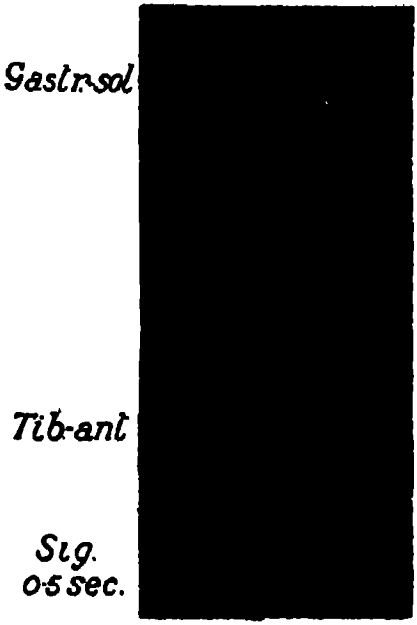

FIG. 11.—Myograms obtained by unipolar faradisation of ipsilateral *n. fastigii*. Sec. dist. 145 mm. (see fig. 2, Plate 17).

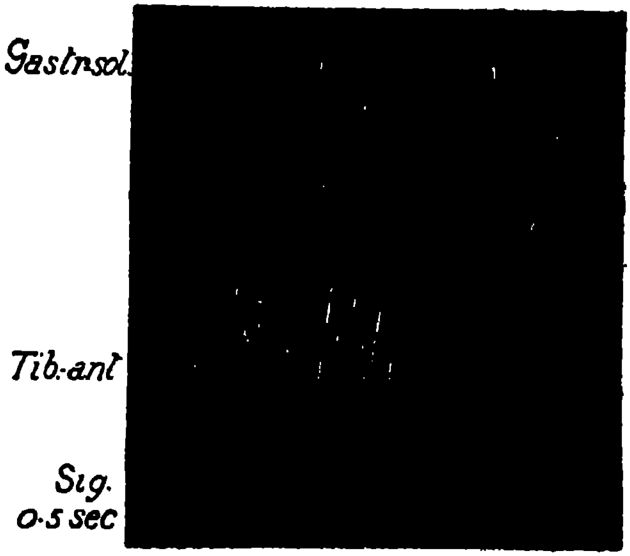

FIG. 12.—Myograms obtained by unipolar faradisation of ipsilateral *n. fastigii*. Movements of progression. Sec. dist. 145 mm.

activity must be considered as infracerebellar in both cases, being spinal and prespinal in location (18).

A phenomenon met with very generally in the course of stimulations of the various nuclei is that of “facilitation” (39), it evidences itself by a progressive increase in briskness of the nuclear responses, with, at the same time, an enhancement of their various phases

Discussion.

The efferent pathways employed by the reactions evokable from the dentate, emboliform and globose nuclei pass forwards from these nuclei in the *brachium conjunctivum*, crossing the midline in Wernekink's commissure and giving off a descending tract destined for the reticular formation of the pons and *medulla oblongata* the fibres end in synapses around the cells of the *nucleus ruber* The majority of the axons from the dentate nucleus probably end in the small celled component of the *n ruber* (*n ruber parvicellulatus*), though some end also in the large celled component (*n ruber magnicellulatus*), the latter nuclear division is the chief ending place for the axons from the emboliform and globose nuclei (13, 24, 1) *

Taking its origin in the *nucleus magnicellulatus* the rubro spinal tract crosses the midline in the decussation of Forel and extends downwards giving off collaterals around the motoneurones of the cord, it is probably of major importance in the lateral nuclear reactions, though those from the dentate nucleus are subserved mainly by the *nucleus parvicellulatus* and its extensions the rubroreticular and reticulospinal tracts

As subserving the responses evokable from the *nucleus fastigii* we must look to the tracts variously known as “*fasciculus uncinatus*” (Russell (34)) Hacken bundel” (Probst (31)) “faisceau en crochet’ or “faisceau cérébello bulbaire” (van Gehuchten (12)) Our knowledge of the course and destination of these tracts has gained much in precision at the hands of Mussen (29) who succeeded in making very small nuclear lesions with the aid of Clarke's stereotaxic instrument Two efferent tracts from the roof nucleus are designated by Mussen as of major importance, the “hook bundle” and the fastigio Deiters' bundle,” the first crossed, the second uncrossed The hook-bundle originates from the cells of the roof nucleus and, as well, according to Mussen, from those of the *n globosus*, after traversing the midline it passes beneath the opposite roof nucleus loops around the *brachium conjunctivum*, passing medially to the *corpus restiforme*, to the *contralateral* Deiters' nucleus where most of its fibres terminate, the other tract the fastigio-Deiters' bundle (uncrossed) which arises in the roof and globose nuclei passes through the juxta restiform bundle on the *same* side to the *ipsilateral* Deiters nucleus its principal termination Mussen s description includes a number of other fastigiobulbar tracts but those referred to here being crossed and uncrossed lend themselves well to the explanation of the bilateral character of the fastigial response in which both forelegs participate with powerful flexion (28) The vestibulospinal tract forms the final link to the cord

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* It should be stated here that Mussen (29), who studied degenerations after very small nuclear lesions, believes that the *n globosus* contributes fibres to the fastigiobulbar tracts

Comparing the nuclear reactions in the fore and hindlimbs there is clearly much similarity in the behaviour of the flexors the *biceps brachii* and *tibialis anterior* and in that of the extensors the *triceps brachii* and *gastrocnemius soleus* Generally speaking too there is close resemblance between the individual reactions of the several nuclei as manifested in flexor contraction and extensor inhibition the latter followed by rebound thus the reactions of the lateral nuclei are much alike nor do they differ materially except in being somewhat less intense from those of the *n fastigii* though the efferent pathways from the latter nucleus are separate and distinct both as to course and mode of termination This essential similarity between the functional activities of the various nuclei finds its explanation in their intimate linkage and still more in their common origin and morphological equivalence (29)

In both fore and hindlimb the regulation of muscular antagonists is as stated in conformity with the law of reciprocal innervation (39) We may proceed, then, to a discussion of the reactions with special reference to those of the hindlimb, partly because of their essential similarity to those of the forelimb but more especially because of our greater knowledge of the hindlimb reactions, both reflex and motor cortical thus affording us a wider field for fruitful comparisons and inferences

A characteristic attaching to the responses of *n fastigii* and exemplified by the myograms of both *biceps brachii* and *tibialis anterior* is the promptness and extensiveness of its inception (figs 5 and 11) One is impressed with the resemblance of the steep ascent to the *réaction d'emblée* of Liddell and Sherrington (20), by this designation they refer to the smartness of the rise in the ipsilateral flexor reflex, attributing it to the sudden calling into action of the effective motoneurones The slower rise of the ascent curve evokable from the lateral nuclei, especially *n dentatus* (figs 1 and 7) recalls the " recruitment reaction ' of Liddell and Sherrington (20), thus they interpret the slowly rising phase of the crossed extensor reflex, attributing it to the progressive recruitment of motoneurones (19) One is tempted at first sight to ascribe the type of nuclear reaction to the mode of activation of the motoneurones of midbrain *medulla oblongata* and spinal cord; thus in the fastigial reaction there would be immediate and practically simultaneous activation of all the motoneurones, whilst in the dentate reaction the activation would be gradual and progressive. But other factors come to mind as, in all likelihood, of greater importance: when the electrode is applied over the *n. fastigii* the current probably impinges on a greater wealth of neurones than when it is applied over the more laterally placed nuclei; particularly in the case of the dentate nucleus conditions are least favourable for stimulation, because of its situation at the extreme outer boundary of the nuclear mass. Thus in the case of the roof nucleus there is an immediate excitation of a large number of neurones and, correspondingly, there results a prompt and vigorous response, like a *réaction d'emblée*. But with stimulation of the sparser neurones of the dentate nucleus the muscular activity will tend to increase progressively as more neurones come under the influence of the current through the establishment and extension, by diffusion, of adequate ionic concentrations (30, 14), if we assume that Nernst's theory is applicable to stimulation of the neurones under consideration.

Comparing the behaviour of the hindlimb muscles on nuclear stimulation with that in the ipsilateral flexor reflex, one is struck by the still more marked steepness in the ascent curve of the *tibialis anterior* in the reflex than in the nuclear reaction (figs. 13 and 11). Clearly the activation of the motoneurones

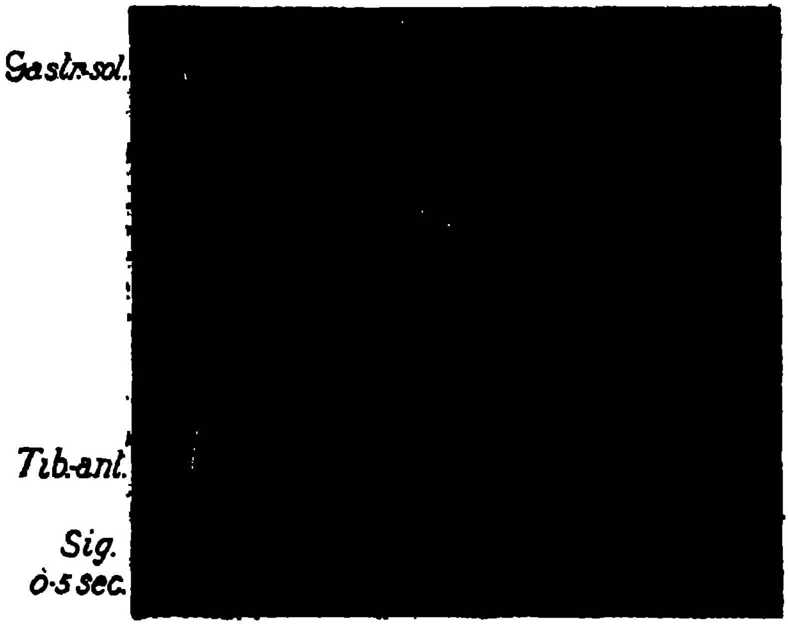

FIG. 13.—Myograms in flexor reflex obtained by faradisation of *nervus tibialis* at ankle. Sec. dist. 90 mm.

is prompter and more general in the reflex than in the nuclear reaction; this we may attribute to the simplicity of the synaptic connections of the reflex arc as compared with those in the pathways leading from the cerebellar nuclei.

The powerful after-discharge of the *tibialis anterior* in the flexor reflex (fig. 13) forms a remarkable difference from the nuclear reactions. in which, as was stated, the absence of this component is particularly striking both in the *tibialis anterior* and in the *biceps brachii*. After-discharge appears as a familiar feature in many reflexes (the stretch reflex excepted), the muscular contraction phase being greatly prolonged as compared with that yielded by direct stimulation of the motor nerve (39, 43). Pronounced after-discharge is an element further of reactions evoked from the cerebral motor cortex, although lacking in those from the subjacent white matter (35, 39).

Explanations of after-discharge put forward by Sherrington attribute it either to the persistence of excitation at synapses or to the traversing in part of reflex pathways more circuitous and complex than those forming the most direct route; that a relationship exists between after-discharge and conduction through devious reflex arcs is suggested by the observation of Bremer and Rylant (4) that strychnine obliterates the after-discharge in the reflex electromyogram by its tendency to equalise conduction rates through the different arcs.

The influence of varied synaptic connections and of dissimilar speeds of transmission within the cerebral cortex may with reason be considered operative in causing after-discharge when stimulation is applied to the cortical surface; but, admitting asynchronism in synaptic conduction in the cerebral cortex, we must reason that such does not exist, or must be minimal, at the spinal levels of connection of the corticospinal fibres, since subcortical excitation of these fibres does not yield after-discharge.

If we seek to explain the absence of after-discharge in the cerebellar nuclear response in like manner, as due to comparatively simple synaptic connections, we must admit the existence in the descending cerebellar tracts of greater synaptic complexity than in the continuous pathway of the pyramidal fibres; thus, whilst the final terminations of the cerebellar pathways in the cord may be quite as simple as those of the pyramidal fibres, the synaptic connections in midbrain and *medulla oblongata* are somewhat intricate; apparently, however, these junctions permit of an even and regular transmission of impulses, so that after-discharge fails to appear.

As bearing on this problem the fact must be mentioned that in the absence of after-discharge the stretch reflex exhibits a point of resemblance to the cerebellar nuclear reactions and the idea naturally comes to mind as to whether any factors of similar nature may be operative in both cases; but, regarding this possibility, further investigations are needed to determine whether the resemblance has any underlying significance.

A prominent characteristic of the myograms of *triceps brachii* and *gastrocnemius-soleus* is inhibitory relaxation during the period of nuclear stimulation; the nuclear inhibition is, as already stated, clearly identical with that evokable by faradisation of various parts of the cerebellar cortex, including the rostral surface and the median parts situated farther caudally (36, 27). These cortical areas are connected with the *n. fastigii*, *globosus* and, to some extent, with the *n. dentatus*, though this latter also receives fibres from the lateral cortex (7, 15), regarding the responses of which we have as yet insufficient knowledge; still there can be no real doubt as to the identity of inhibitions evokable from cortex and nuclei. The nuclear or cortical inhibition, each exemplifying one and the same reaction, is obviously allied to the inhibition of tone elicitable reflexly in an extensor muscle (fig. 13).

In particular, cerebellar inhibition resembles the inhibition of a myotatic extensor response evokable by traction on the antagonistic flexor muscle (22). Like this latter expression of reflex inhibition that from the cerebellum, as tested from the cortex, is "pure," that is, it is unmixed with any positive or augmentor component of influence over the neurones of the inhibited muscles; thus strychnine on intravenous injection (3) or local application (26) to the cortex does not obscure the inhibition through the intensification of latent motor potentialities.

Both cerebellar and reflex inhibition require for their demonstration some degree of pre-existing tone and both are succeeded by prompt and powerful "rebound" contractions (38, 40, 5). Like reflex rebound cerebellar rebound is central, due to recoil from inhibition to excitation, and is not conditioned merely by the actual lengthening of the muscle in the precurrent inhibition; thus the muscular lengthening may be slight or even absent and yet the rebound may be intense, as in the case of the reflex (40); these facts are illustrated in the case of the *triceps brachii* on stimulation of the *n. globosus* in fig. 4 and of the *n. fastigii* in fig. 5; in each case, though the precurrent muscular relaxation is slight, the rebound is powerful, leading to greater tone than that of the resting muscle. In prompt incidence and gradual subsidence cerebellar rebound resembles reflex rebound. One is inclined to regard the phenomena of "release" after cerebellar ablation (Luciani's first stage) as closely allied to those of cerebellar rebound; that is, removal of the cerebellum results in a condition essentially equivalent to that which ensues on cessation of cerebellar stimulation.

So far as the muscles of the trunk are concerned we have only observed their responses incidentally, but they appear to be homologous with those of the limbs; thus, during stimulation of the nucleus there is ventral flexion of the body and after stimulation, dorsal flexion or extension, appearing as a powerful rebound. Thus cerebellar nuclear stimulation evokes augmentation of tone in the flexor muscles with inhibition of tone in the antigravity muscles of both limbs and trunk, to be succeeded by powerful tonus rebound in the antigravity group. The two antagonistic muscle groups thus come within the realm of positive cerebellar influence, the flexor group during the period of cerebellar activity, the antigravity group thereafter. Whilst this statement seems to embody the general principles of cerebellar control, we must state that we have seen, in a few instances, extension of the ipsilateral forelimb take the place of flexion during stimulation of the *n. emboliformis* (28); for this deviation we have as yet no explanation, and flexion, not extension, appears to be the rule during the course of stimulation.

The observation of de Barenne (2) and Rademaker (32) that hypotonia does not develop after cerebellar ablation was discussed in the introduction and was shown to offer no difficulty for our view as to the dual nature of cerebellar control over postural tone. We can easily picture the cerebellum as influencing the postural tone of the living organism, in accordance with the principles outlined above. For instance, in the case of an animal changing from the standing to the crouching posture, the cerebellum would be called into activity so as to assist in augmenting the tone of the flexor muscles, whilst at the same time inhibiting the tone of the antigravity muscles; the reassumption of the standing posture would be furthered by the contribution of cerebellar rebound to the antigravity muscles.

Extending these conceptions to human beings, we may consider a man performing gymnastic exercises consisting in successive squatting and standing; the first would correspond to the cerebellar flexor excitation phase, accompanied by inhibition of the antigravity muscles, the second or erect posture to the phase of cerebellar rebound manifesting itself by increased tone in the antigravity muscles. Both kinds of cerebellar influence would be superimposed on the primary activities of reflex and voluntary origin. Very reasonable is the assumption of similar cerebellar contributions to the act of walking; thus the phase of cerebellar excitation would contribute to the movement of flexion, the phase of cerebellar rebound to that of extension. These cerebellar influences over tone we must conceive as being superimposed on the basic activities of progression, themselves determined in the spinal, prespinal or cerebral cortical centres. In the disturbance of these cerebellar influences we may seek the explanation not only of cerebellar *ataxia* but of such a symptom as *dysmetria*.

Our observations lead us to conclude that the influence of each cerebellar nucleus extends to the greater portion of the muscles, being most pronounced ipsilaterally, whilst the control over antagonistic groups is strictly reciprocal and co-ordinated. But the fundamental co-ordinating power resides not in the cerebellum but in the various centres, spinal and prespinal in location; to the fundamental, though crude, activities of these centres the augmentor, inhibitory and rebound influences on tone emanating from the cerebellum are superadded, thus contributing elements of accuracy and refinement to the complex phenomena of movement and posture.

Summary.

(1) Myograms of muscular antagonists in the fore- and hindlimb of the decerebrate cat were secured on subjecting the ipsilateral cerebellar nuclei to unipolar faradisation.

(2) The *nucleus dentatus* yields, during stimulation, increase of tone in the *biceps brachii* and *tibialis anterior* together with inhibition of tone in the *caput laterale* of the *triceps brachii* and *gastrocnemius-soleus*. Following stimulation there is immediate relaxation, devoid of after-discharge, in *biceps* and *tibialis*, together with tonus " rebound " in *triceps* and *gastrocnemius-soleus*.

(3) *Nucleus emboliformis* and *n. globosus*. The muscular responses evokable from these nuclei are closely similar and are of the same kind as those from the *n. dentatus*, though they are more intense. The increase of tone of *biceps brachii* and *tibialis anterior* during the stimulation is vigorous, whilst the subsequent relaxation occurs without after-discharge. The inhibition of *triceps brachii* and *gastrocnemius-soleus* is pronounced and is followed by extensive rebound.

(4) *Nucleus fastigii*. The myograms from this nucleus resemble those from the other nuclei though the various phases are still intenser than in the case of *n. emboliformis* and *globosus*.

(5) The great intensity of the responses evokable from the more medially placed nuclei, particularly the roof nuclei, is attributed to the wealth of neurones, which come under the influence of the current when the electrode is applied above these nuclei.

(6) The responses of antagonistic muscles in fore- and hindlimb are co-ordinated, showing adherence to the principle of reciprocal innervation; the fundamental co-ordinating mechanisms are regarded as being infracerebellar in location.

(7) The efferent pathways for reactions from the lateral nuclei consist of *brachium conjunctivum*, *nucleus ruber* (*parvicellulatus* and *magnicellulatus*), rubroreticular and rubrospinal tracts. The pathways from the roof nuclei are the "hook-bundle" and the "fastigio-Deiters' bundle."

(8) The nuclear responses resemble the ipsilateral flexor reflex in respect to the inhibitory relaxation followed by rebound in the case of the antigravity muscles. These two phases are considered as being of like modality in nuclear responses and in the reflex. The nuclear inhibition and that evokable from the cerebellar cortex are regarded as being identical. The flexor motoneurones show prompter activation in the flexor reflex than in the briskest nuclear response. After-discharge is lacking from the nuclear responses in the case of the flexor muscles.

(9) The cerebellar nuclei, on being excited, emit impulses, which augment the activity of the motoneurones of the flexor muscles, whilst inhibiting that of the motoneurones of the antigravity muscles; after the excitation the tone of the latter muscles is intensified through "rebound." Cerebellar control is thus of a *dual* nature.

(10) In daily life similar influences are exerted, flexor tone being increased, and antigravity tone decreased, by cerebellar activity, whilst antigravity tone is augmented through rebound on suspension of the cerebellar discharge; thus, for instance, cerebellar excitation would contribute to the flexor phase of the step, cerebellar rebound to the extensor phase. Disorganisation of this dual cerebellar control through morbid processes leads to *ataxia* and such symptoms as *dysmetria*.

Mr. T. W. Stewart, assistant in the department, executed the histological and photographic work for this paper.

DESCRIPTION OF PLATES 17 AND 18.

Figs. 1, 2, 3 and 4.—Sections (oral surfaces) of *cerebellum* and *medulla oblongata* of cat, showing position of unipolar electrode over the cerebellar nucleus in each experiment. El. dent.; El. embol.; El. fastig.; El. glob.: electrode over *nucleus dentatus*, *n. emboliformis*, *n. fastigii* and *n. globosus* respectively. *Nuc. dent.*: *nucleus dentatus*.

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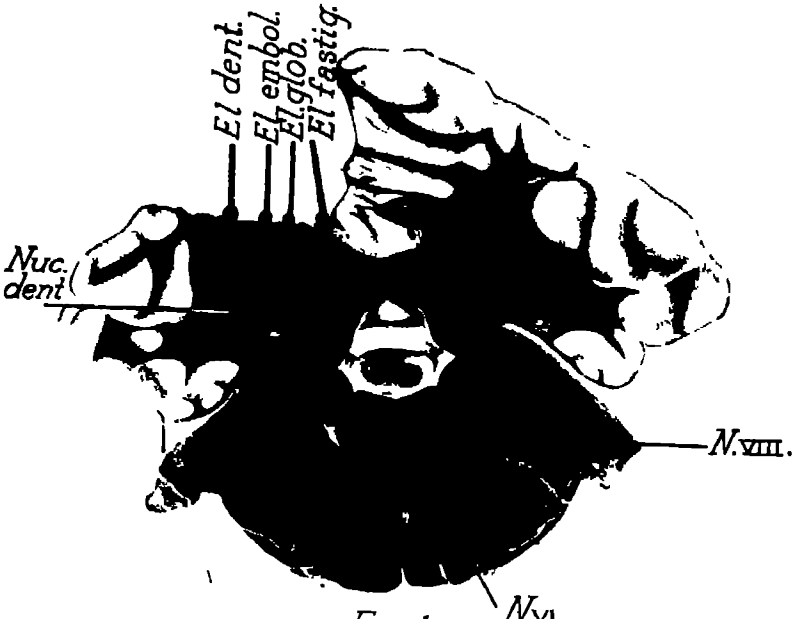
El dent.
El embol.
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Nuc. dent.
N. VIII.
N VI

Fig. 1.

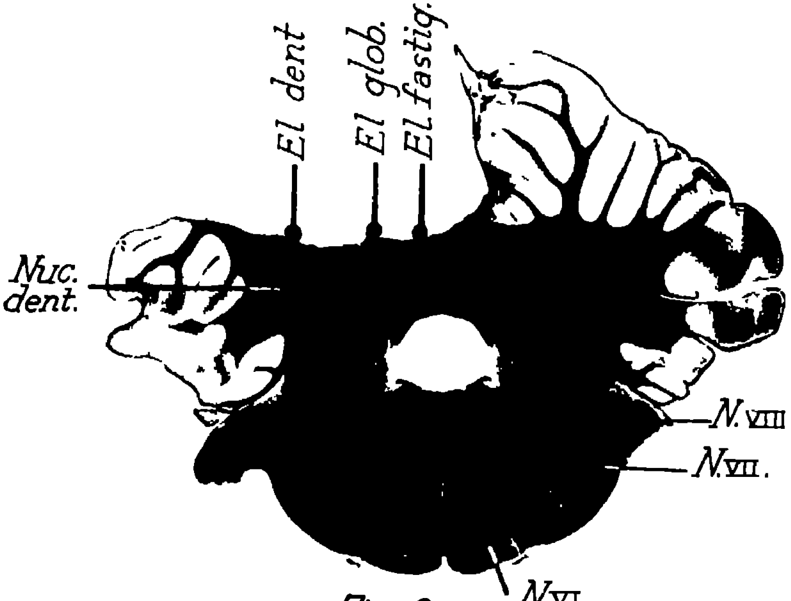
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Fig. 2.

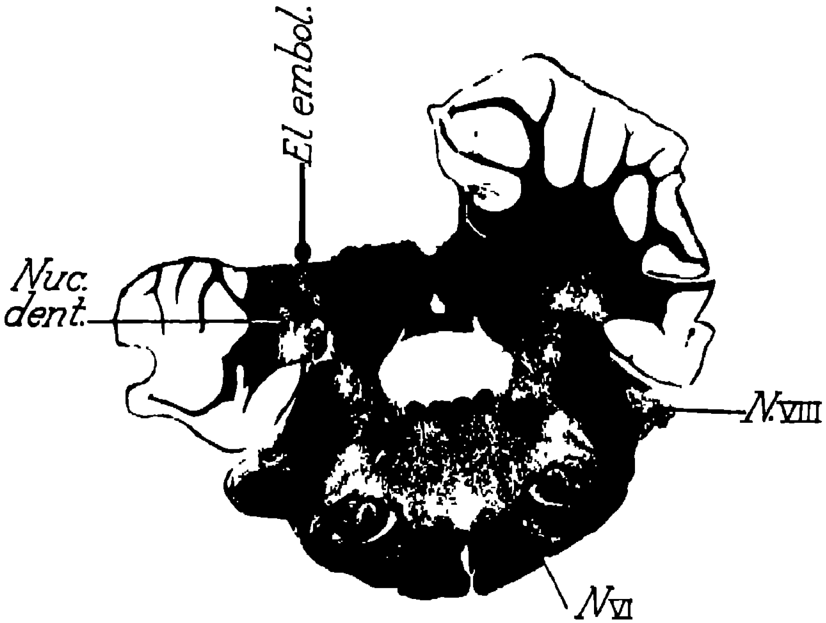

Fig. 3.

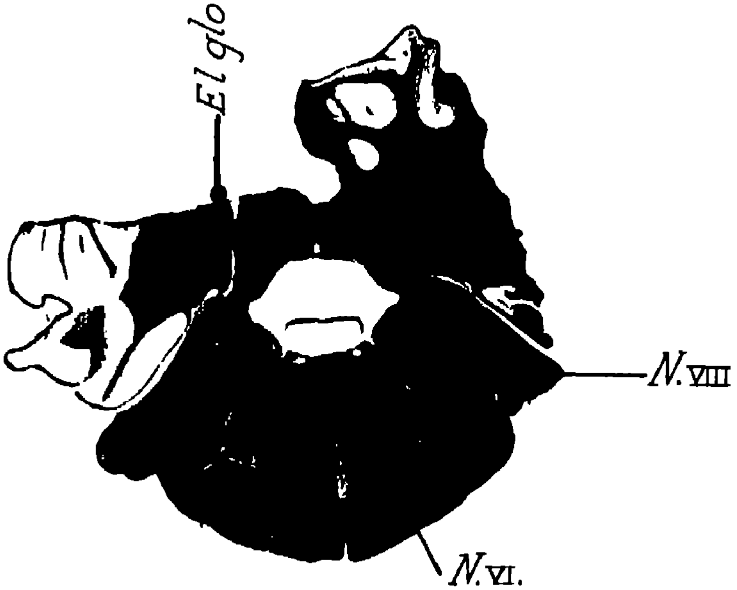

Fig. 4.

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Further Observations on Chinese Kala Azar.

By Edward Hindle, Kala Azar Commission of the Royal Society.

(Communicated by H. H. Dale, Sec.R.S.—Received September 11, 1928.)

[Plates 19-20.]

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General Account.

The results of our experiments at Tsinan, in 1926, on the development of flagellates in sandflies fed on infected hamsters, and also on human cases of Chinese Kala Azar (Patton and Hindle, 1927), indicate that both *Phlebotomus major* var. *chinensis* and *P. sergenti*, but especially the former, are favourable hosts for *Leishmania*.

During the summer of 1927 these experiments were continued, employing the same methods as those described in the previous reports of our Commission. Unfortunately, owing to political disturbances, the laboratory had to be moved to Tsingtao, on the coast, where the only sandflies we succeeded in finding were *Phlebotomus taianensis* (Patton and Hindle, 1928), and in one house odd specimens of *P major* var *chinensis* Consequently it was necessary to establish a collecting station in some region where sandflies could be obtained in greater numbers and eventually Wei hsien about 100 miles from the coast was selected as the most convenient place *

The sandflies were bought from the local inhabitants and were at once sorted into breeding pots or feeding boxes according to whether they contained undigested blood or not Each day the sandflies were sent to Tsingtao by train and this method worked fairly satisfactorily so long as the package was not overheated as for example by being exposed to the sun as under these conditions the flies very soon died Unfortunately a local rebellion interrupted communications between Wei hsien and Tsingtao for a fortnight at the height of the *P major* season and curtailed the number of experiments we were able to make with this species

The flies were fed in the manner we have previously described with the exception that all the hamsters used for feeding experiments were given an injection of urethane previous to the operation This preliminary narcotisation was found to produce more satisfactory results as by its use the flies when feeding were not disturbed by any movements of the animal

The results of our experiments in 1927 confirm the observations on the type of development of Chinese *Leishmania* in the two Chinese species of *Phlebotomus* that feed on man In the case of *P major* var *chinensis* the flagellates become attached to the wall of the mid gut and usually within three or four days of the infective feed may be seen lining the narrow anterior region adjoining the œsophagus In a certain proportion of sandflies the flagellates pass forward through the œsophagus and then multiply in the pharynx and extend down the proboscis Invasion of the pharynx when present usually takes six days from the infective feed although occasionally it was observed after five days The persistence of flagellates in the gut of *P major* does not require the presence of undigested blood as in many instances flies which had completely digested their last meal contained large numbers of flagellates attached to the lining of the mid gut

P sergenti seems to be an equally favourable host for the early development of Chinese *Leishmania* but in this species the flagellates remain confined to the broad posterior region of the mid gut and do not become attached to the lining of the alimentary canal Also invasion of the œsophagus or pharynx has never been observed. The persistence of flagellates in the mid-gut seems to be dependent on the presence of undigested food material, unlike *P. major*, and consequently when the alimentary canal does not contain any food material the flagellates disappear. Many examples are given below in which batches of *P. sergenti* were fed simultaneously on an infective animal, and when dissected after four days' incubation period all flies that still contained undigested blood in the mid-gut were positive, and those which had completely digested the meal were negative.

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* We should like to express our thanks to Dr Fwers of the American Presbyterian Mission for much valuable assistance in the establishment of this station and help in obtaining cases of kala azar

The examination of between three and four thousand sandflies has shown that the above types of development are very constant, and although in one or two instances flagellates were seen in the hind-gut and once in the diverticulum, these were such obvious exceptions that their presence was probably the result of accidents. Although carefully looked for, no trace of intracellular development in the wall of the alimentary canal was ever observed, nor any invasion of the salivary gland. In *P. sergenti* the infection remains confined to the mid-gut, whilst in *P. major* var. *chinensis* the flagellates, in addition, extend forward and may invade the pharynx and proboscis.

Feeding Experiments on Human Patients.

During 1927 only four patients were used for feeding experiments, as partly owing to the widespread disturbances and also to the general distrust of new treatment, it was almost impossible to persuade anyone to come to the laboratory.

Kala Azar has not yet been recorded from Tsingtao, and although a tour of the neighbouring villages was made, no cases were found in the immediate vicinity About 60 miles to the north and north-west, however, many villages were found to be infected, and in particular a town, Nan-Tsun, where, in a brief survey, numerous cases were observed. Also this place had a record of numerous deaths from Kala Azar, and the local official was very desirous of our opening a treatment centre in the town. As this was not possible, with great difficulty we managed to persuade four patients to come into Tsingtao for treatment. The results of feeding sandflies on these patients, who all had well-marked symptoms of the disease, are given below, from which it will be seen that two gave positive results and two negative.

Case 1.—This patient, a woman, stated that she had had the disease for 24 years, but recently the symptoms had become worse. Treatment with intravenous injections of "Stibosan" for a period of 5 weeks produced an uninterrupted recovery A total of 44 *P. major* var. *chinensis* and 160 *P. sergenti* were fed on this patient on four separate occasions, and dissected after suitable intervals with entirely negative results

Case 2 —This patient, a boy about 11 years old, first showed signs of the disease in April, 1927, and came to the laboratory on July 1 Sandflies were fed on six occasions with the following results —

July 1 —Fed 49 *P sergenti* and 12 *P major*, which were dissected after intervals of 4 and 6 days respectively One *P major* showed a very slight infection of the mid gut but all the other sandflies were negative

July 2 —14 *P major* fed and dissected after intervals of 4, 5 and 8 days One individual dissected after 5 days showed a few flagellates in the mid gut, which were inoculated into a hamster with negative results The other flies were all negative

July 4 —11 *P major* fed and dissected after intervals of 2, 5 and 6 days One individual, dissected after 6 days was positive in the mid gut, the others were all negative

July 16 —11 *P sergenti* fed and dissected after 4 days were all negative

July 18 —8 *P sergenti* fed and dissected after 3 days interval Six flies were negative and two positive in the mid gut The contents of these flies were inoculated into a giant hamster, with negative results

July 19 —21 *P sergenti* fed and dissected after 3 days' interval All negative

It will be seen that a total of 89 *P sergenti* and 37 *P major* were fed on this patient and in only two of the former and three of the latter species was there any subsequent development of flagellates

Case 3 A woman about 40 years of age in a very advanced stage of the disease Treatment with Stibosan " was begun on July 5 Sandflies were fed on six occasions as follows —

July 2 —Fed 37 *P major* and 24 *P sergenti* The latter were all dissected after 4 days interval and one was positive in the mid gut, the others were negative The *P major* were examined after intervals of 4 days, 10 negative 5 days, 12 dissected of which 3 positive in mid gut and 9 negative, 6 days 3 dissected, of which 1 positive and 2 negative, 7 days, 12 dissected, of which 3 positive and 9 negative The contents of the positive flies were inoculated intraperitoneally into three hamsters with negative results

July 4 —30 *P sergenti* fed and dissected after an interval of 4 days All negative

July 16 —2 *P sergenti* fed and dissected after 3 days' interval Both negative

July 17 —13 *P sergenti* fed and dissected after 3 days interval All negative

July 19 —17 *P sergenti* fed and dissected after 3 days' interval 16 were negative and 1 positive in the mid gut, which was inoculated into a hamster with negative results

August 1 —29 *P sergenti* fed and dissected after 3 days' interval All negative

A total of 37 *P major* were fed on this patient, of which 7 subsequently showed development of flagellates in the mid-gut, and 115 *P sergenti*, of which only 2 were positive, but many of these flies were fed after the treatment had begun.

Case 4. - A total of 80 *P. sergenti* were fed on this patient, a woman, on three separate occasions, and in each case dissected after an interval of 3 days. All the flies were negative.

If we compare the results of feeding sandflies on patients infected with the Chinese strain of Kala Azar with those infected with the Indian strain, a great difference is observed in the number of flies which subsequently show development of flagellates. Young and Hertig (1926) in their experiments obtained entirely negative results when *P. major* were fed on human cases, and Young has used these negative results as an argument against the view that sandflies are responsible for the spread of the disease in China

Although we obtained positive results in both 1926 and 1927 by feeding sandflies on human patients infected with Kala Azar, only 4 out of 14 patients were infective to these insects, and even in these 4 the proportion of sandflies that showed any subsequent development of flagellates was only about 5 per cent. It is evident, therefore, that the Chinese strain of *Leishmania* is less infective to *Phlebotomus* than the Indian strain. This difference seems to be correlated with a lower degree of virulence, for in human beings the Chinese strain produces a more chronic type of disease than the Indian strain of Kala Azar.

Feeding Experiments with Hamsters infected with Chinese Kala Azar.

The results of feeding sandflies on human patients, all in advanced stages of the disease, gave such a large number of negative results that in 1927 especial attention was directed towards the influence, if any, of the nature of the infection in the vertebrate host. Consequently the infected hamsters were not specially selected as in the previous year, as we wished to find out what factors influenced the development of flagellates in sandflies that had ingested the blood of animals suffering from leishmaniasis.

The results of our experiments with both *P. major* and *P. sergenti* are summarised in Table I, which gives the number of each hamster, the size of the spleen, the number of parasites in the liver, spleen and bone marrow respectively, and the duration of the infection from the date of inoculation to the date of the first feeding experiment. In the case of *P. major* records are given of the number showing infection of the pharynx. *P. sergenti*, which was the only species available for the later experiments, is not such a good test for infectivity as *P. major*, since the flagellates die out as soon as the

Table I

Number of hamster	Duration of infection (in months)	Number of parasites in organs of hamster			*P major*		*P sergenti*		Remarks
		Liver	Spleen	Bone marrow	Total number fed	Number infected	Total number fed (less empty ones)	Number infected	
126	15	++	++	++	23	7	12 (−3)	7	
140	15	++++	++++	++++	31	17	13 (−4)	7	
141	15	+++	+++	+++	84	81	44 (−13)	27	Six *P major* positive in pharynx Three inoculation experiments positive
146	15	++	+++	+	5	0	11	0	
155	15	Neg	+	Neg	2	0	22	0	
158	15	++	++	+	69	66	133 (−37)	96	Seven *P major* positive in pharynx
162	15	++	++	+++	—	—	14	0	
173	15	+++	+++	+++	1	1	—	—	Positive in pharynx
260	14	+++	++++		2	1	35 (−53)	41	
303	14	+++	++	++	—	—	19 (−13)	2	
323	13	++++	++++	++++	2	1	—	—	Positive in pharynx
331	13	++++	++++	++++	100	96	84 (−23)	59	Details of this experiment are given below
382	13	++++	++++	++++	47	38	40 (−18)	23	Four *P major* positive in pharynx One inoculation positive
644	8	+	++	++	14	0	6	0	
653	8	++++	++++	++++	10	0	9	1	
655	8	+++	+++	+++	2	2	40 (−25)	14	
658	8	++	++++	++	2	0	24 (−18)	0	
672	8	++	++++	++++	9	4	70 (−21)	34	
673	8	+	+++	+++	8	1	2	0	
690	8	Not examined			22	9	10	2	One *P major* positive in pharynx
693	8	+	++++	++	2	1	9	3	
712	8	Neg	Neg	Neg	—	—	23 (−8)	0	
737	8	++++	++++	++++	23	21	44 (−7)	37	Seven *P major* positive in pharynx
738	8	++++	++++	++++	32	19	46 (−12)	25	Two *P major* positive in pharynx Two inoculations positive
753	6	++++	++++	++++	17	9	25 (−2)	23	
756	6	++++	++++	++++	7	0	36 (−25)	11	
758	16	++++	++++	++++		—	12	6	
759	6	+++	++	++++	5	2	31	1	
760	6	+	+	+	10	3	12	4	
800	6	+	Neg	+	—	—	26 (−13)	5	Skin positive
801	6	+++	++++	+++	13	11	19 (−11)	8	Three *P major* positive in pharynx
802	6	++++	++++	++++	2	1	24 (−14)	10	Skin heavily infected
803	6	++++	++++	++++	—	—	22 (−15)	4	
823	3½	+	+	+++	2	1	22 (−19)	2	
826	3½	+	+	+	—	—	27	0	
828	3½	++	++	++	—	—	19 (−9)	0	Skin negative
829	3½	++	++	+++	—	—	27 (−11)	5	
830	3½	++++	++++	++++	14	4	27 (−12)	13	Skin positive
831	3½	++++	++++	++++	—	—	21 (−18)	1	
832	3½	+	+	Neg	—	—	30 (−19)	1	
834	3½	++	++	++	—	—	12 (−3)	2	
836	3½	++++	++++	++	—	—	35 (−12)	2	
837	3½	++	+	+	—	—	9 (−3)	0	
839	3½	++++	++	+++	—	—	31 (−16)	5	
841	3½	++	++	+	—	—	31 (−11)	2	
843	3½	+++	+++	+	—	—	32 (−22)	2	
845	3½				—	—	31 (−9)	17	
846	3½	++++	++++	++++	—	—	28 (14)	7	
847	3½	+++	++++	++++	—	—	15 (−8)	0	
849	3½	+	—	Neg	—	—	10 (−5)	0	
851	3½	+	++	++	—	—	12 (−6)	1	
853	3½	++++	++++	++	—	—	11	0	
854	3½	++	+	+	—	—	19 (−10)	1	
855	3½	—	Neg	Neg	—	—	20 (−15)	2	
856	3½	++++	++++	++++	—	—	18 (−7)	0	Skin negative
857	3½	++	++	+++	—	—	32 (−20)	0	
858	3½	Neg	+	Neg	—	—	21 (−11)	0	
859	3½	+++	+++	+++	3	0	—	—	
860	3½	++++	++++	+++	—	—	18 (−12)	0	Skin negative
861	3½	+	+	Neg	—	—	18 (−10)	0	
863	3½	++++	++++	++++	—	—	27 (−18)	0	
864	3½	++++	++++	+++	—	—	12 (−9)	0	
865	3½	+	+	+	—	—	14 (−10)	0	
866	3½	Neg	+	Neg	—	—	23 (−9)	0	
867	3½	++	++	++	—	—	11	0	
868	3½	+	+++	+	—	—	21 (11)	2	
869	3½	++++	+++	++++	—	—	19 (−12)	1	
870	3½	++	++	++	—	—	10 (4)	0	
873	3½	+	+	+	—	—	30 (−14)	0	
875	3½	—	+	Neg	—	—	13 (−5)	0	
876	3½	—	+++	+	—	—	46 (−28)	0	
877	3½	+	++	+	1	0	9 (−6)	0	
879	3½	+	+	Neg	—	—	18 (−9)	0	Skin negative
883	3	++++	++++	++	—	—	14 (−9)	0	Skin negative
913	1½	—	+	+	—	—	41 (−25)	0	
932	1	+++	+++	+++	—	—	54 (−30)	16	Skin negative

blood is digested. Consequently, it is necessary to strike a mean between the most favourable period for the development of the flagellates, and the time taken to digest the blood. In practice, the flies were left for 3 or 4 days and when dissected any that contained no trace of food material (which were invariably negative) were deducted from the total number. In Table I the number of these empty flies is indicated in brackets after the total number dissected.

Hamster No. 331.

The results of feeding experiments with this hamster are given in detail as it represents one showing a very high degree of infectivity to sandflies. The animal was inoculated intraperitoneally on May 10, 1926, with flagellates from a 4-day old culture. Eight months later parasites were found on liver puncture, and on May 20, 1927, parasites were also found in the peripheral blood. Subsequent examinations of the blood in June were sometimes negative and sometimes positive.

Phlebotomus major were fed on this animal on various dates, with the following results. -

June 10.- 7 fed and 1 dissected after 2 days and the others after 6 days. All heavily infected and the 6 individuals dissected after 6 days all showed anterior growth of the flagellates. One fly showed a heavy infection in the pharynx extending to the tip of the proboscis. The contents were inoculated into 3 hamsters with negative results.

June 15.—19 fed and dissected after intervals of 3, 5, 6, 7 and 8 days, respectively. With one doubtful exception all the flies were heavily infected with flagellates, and 15 dissected on the seventh and eighth days all showed infection of the pharynx and proboscis. The contents of these flies were inoculated into 5 hamsters with negative results.

June 21.—9 fed and dissected after intervals of 5, 6 and 7 days, respectively. All heavily infected up to the œsophagus but not in the pharynx. Contents inoculated into 3 hamsters with negative results.

June 25.—16 fed and dissected after intervals of 5 and 6 days. Fourteen were heavily infected, 4 in the pharynx, and 2 were negative. The contents of these flies were inoculated into 3 hamsters and one of these, inoculated intraperitoneally with flagellates from 4 infected flies fed 6 days previously, became infected with *Leishmania*. The other two were negative.

July 1.—30 fed and dissected at intervals of 3 to 9 days. Omitting obviously degenerate examples all were heavily infected, and from the seventh day onwards 3 out of 16 were positive in the proboscis. Their contents were inoculated into 5 hamsters and the flies re-fed on normal animals with negative results.

July 8.—6 fed and dissected after intervals of 4, 5 and 6 days were all positive. The contents of one were inoculated into a hamster with negative results.

July 16.—3 fed and dissected after 5 days' interval. All positive, one of them showing flagellates in the pharynx. These were inoculated into a giant hamster with negative results.

July 23.—5 fed and dissected after 3 and 4 days' interval. All positive, but not in pharynx. The contents were inoculated into 2 hamsters with negative results.

July 27.—2 fed and dissected after 3 and 5 days' interval, respectively. The latter was heavily infected in the œsophagus; the contents were inoculated into 2 hamsters with negative results.

August 1.—3 fed and dissected after intervals of 3, 4 and 7 days. Two positive and one negative, but degenerated. The contents were inoculated into two hamsters also the flies re-fed on a normal hamster with negative results.

Summarising the results of these experiments it will be seen that 100 *P. major* were fed on this hamster, all of which, with one or two doubtful exceptions, showed a heavy infection with flagellates in the mid-gut. In every case the flagellates had grown forward to the anterior end of the mid-gut, and out of 57 flies that were dissected after an interval of 6 or more days from the infective feed, 15 of them, approximately one quarter, showed flagellates in the pharynx and proboscis. Although no hamsters were infected by the bites of these flies when re-fed, in one instance a typical infection with *Leishmania* was produced by the intraperitoneal inoculation of the contents of four infected flies.

Phlebotomus sergenti were fed on this hamster on four occasions as follows:—

June 10.—10 fed and dissected after 2 and 3 days were all positive in the mid-gut. The contents of 8 flies dissected on the third day were inoculated intraperitoneally into a hamster which became infected and was heavily infected when examined 6 months later.

June 15.—39 fed and dissected at intervals of 3 and 4 days. Eleven flies were empty, and consequently negative, but the remaining 28 all contained numerous flagellates. The contents of 27 positive flies, fed 3 days previously, were divided into two portions and one inoculated intracutaneously, and the other intraperitoneally into two hamsters. The hamsters which received the intracutaneous inoculation remained uninfected, whilst the other animal, inoculated intraperitoneally, showed a heavy *Leishmania* infection when examined after an interval of six months.

August 8.—9 fed and dissected after 3 days. Seven positive and two empty ones negative. The contents of one of the infected flies were inoculated into a hamster with negative results.

August 14.—14 fed and dissected after 3 days' interval. Seven positive and 7 empty ones negative.

It will be seen that 72 *P. sergenti* were fed on this animal and, with the exception of 20 empty flies, every individual when dissected showed flagellates in the mid-gut, and in two instances infections were produced in hamsters by the intraperitoneal inoculation of the contents of infected sandflies.

Correlation between Skin Infection and Infectivity to Sandflies.

The certainty with which sandflies became infected when fed on the above described hamster was so remarkable that a careful examination was made of this animal in order to see if any explanation could be found of this high degree of infectivity. The most obvious explanation seemed to be the presence of parasites in the peripheral circulation, and therefore films were made of the blood at the same time as the sandflies were fed. No correlation could be found between the presence of parasites in the blood and the development of flagellates in the sandflies that fed on the hamster. The blood films were negative for *Leishmania* on three occasions, when all the sandflies became infected, and although the blood was positive on four other occasions, the parasites were so rare that their numbers were insufficient to explain the development of such large numbers of flagellates as were present in the insects after only 3 days. Consequently, when the hamster died all its organs were carefully examined and it was found that the sub-dermal layer contained incredibly large numbers of *Leishmania*, in certain regions the infection being so intense as to produce nodules closely resembling those of Oriental Sore (see figs. 1 and 2) A section of a typical part of the skin of this hamster is shown in figs. 3 and 4. The parasitised endothelial cells, clasmatocytes, are seen to have migrated from the peripheral blood vessels into the surrounding tissue and in fig. 3 may be seen arranged concentrically around one of the vessels. It is evident that in feeding, the proboscis of the sandfly must pierce some of these infected cells and the contained parasites be ingested.

In addition to this hamster No. 331, several other animals were examined to see whether there was any correlation between the presence of *Leishmania* in the skin and the number of sandflies that became infected when fed on these hosts The results clearly indicate that there is such a correlation for, with one exception, parasites were found in the sub-dermal tissues of all animals that were infective to sandflies, whilst conversely, no matter however intense the infection of the internal viscera, unless parasites were present in the skin, insects fed on such hamsters remained negative. Hamster No. 800 is an example of an animal with a very slight infection of the liver and bone-marrow, and the spleen was negative, yet 5 out of 13 *P. sergenti* that were fed on it showed development of flagellates. Blood films made immediately after the feeding experiment were negative, but when sections of the skin were examined a moderate infection was found to be present.

Hamsters Nos. 856, 860 and 883 are good examples of animals in which the liver, spleen and bone-marrow were very heavily infected, but which gave negative results when sandflies were fed on them. The examination of the skin in these cases, and also in all others in which the hamster was negative to sandflies, has never resulted in the discovery of *Leishmania* in the subdermal tissues. One individual, hamster No. 922, which had been inoculated only one month previously, gave positive results when sandflies were fed on it, but no parasites could be found in the portion of skin examined by us. The examination of the blood, however, was negative and one is compelled to assume that in this individual the skin infection was very localised and the flies fed on a particularly favourable region.

In view of the undoubted fact that parasites may sometimes be present in the peripheral blood, although in extremely small numbers, the sandflies may occasionally derive their infections from this source, but there is such a marked correlation between the number of parasites in the skin and the proportion of flies becoming infected that in the majority of cases the infection would seem to be derived from the skin

Existence of Strains of Varying Degrees of Virulence.

The extremely variable results obtained by feeding sandflies on hamsters inoculated at the same time with similar doses of infective material is shown in the above table, and clearly indicates the importance of the vertebrate host in producing variations in infectivity towards the insect host. It seemed of interest, therefore, to see if there was any evidence of the existence in nature of human strains of varying degrees of virulence. With this object a number of hamsters were infected by inoculating them with the material obtained by liver puncture of patients suffering from Kala Azar. Five strains were thus isolated, subinoculated into other hamsters, and then tested as regards their infectivity to sandflies.

Human Strain A.—On May 19, 1926, a hamster was inoculated intraperitoneally with liver puncture material from case No. F 1584*, a typical case of Kala Azar in a Chinese patient. Ten months later this hamster was killed and examined for *Leishmania*. The spleen was only moderately enlarged, but the liver, spleen and bone-marrow contained large numbers of parasites. A saline suspension of the liver and spleen of this animal was subinoculated into 12 hamsters, care being taken to give each animal the same dose.

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[1] The case numbers are those of the Hospital of the Shantung Christian University, Tsinan.

The following table indicates the results of the inoculations, and also the percentage of sandflies that became infected when fed on these animals:—

Table II.

Number of hamster	Interval between inoculation and death.	Result of post-mortem examination				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen	Number of parasites in—			
			Liver.	Spleen.	Bone-marrow.	
A 1	5 months	Much enlarged	+	+	+++	60 per cent.
A 2	1 day	—	—	—	—	—
A 3	10 days	Normal	+	+	Neg.	—
A 4	5 months	Enlarged	+	+	+	None.
A 5	15 days	Normal	Neg	Neg.	Neg.	—
A 6	5 months	Much enlarged	++	++	++	None.
A 7	5 months	Enlarged	++	++	+++	31·3 per cent.
A 8	5 months	Much enlarged	++++	++++	++++	87 per cent.
A 9	5 months	Much enlarged	++++	++++	++++	33 per cent
A 10	4 months	Enlarged	+	+	Neg	9 per cent.
A 11	2 months	Enlarged	+	+	+	—
A 12	5 months	Much enlarged	++	++	++	22 per cent.

Human Strain B.—On May 1, 1926, hamster No. 305 was inoculated intraperitoneally with liver puncture material from case No. F 1488, and killed on March 14, 1927. The spleen of this animal was very much enlarged and the liver and spleen contained enormous numbers of parasites. The bone-marrow was only moderately infected. A liver and spleen suspension was inoculated intraperitoneally into 12 hamsters, each receiving an equal quantity of infective material.

The results are indicated in the following table.—

Table III

Number of hamster	Interval between inoculation and death	Result of post mortem examination				Percentage of sandflies showing flagellates when fed on hamster
		Size of spleen	Number of parasites in—			
			Liver	Spleen	Bone marrow	
B 1	5 months	Slightly enlarged	Neg	Neg	Neg	None
B 2	5 months	Enlarged	++++	++++	++	8 7 per cent
B 3	5 months	Enlarged	++	+	+	None
B 4	7 days	Normal	++		Neg	—
B 5	5 months	Enlarged	++++	+++	+++	33 3 percent
B 6	2 months	Enlarged	++	++++	++	
B 7	5 months	Normal	++	+		10 per cent
B 8	2 days	—				—
B 9	5 months	Enlarged	++	+++		20 per cent
B 10	3 months	Enlarged	+	++	Neg	
B 11	3½ months	Enlarged	Degenerated			
B 12	5 months	Enlarged	+	++++	++++	[illegible]0 per cent

Human Strain C—On June 18 1926 hamster No 429 was inoculated intraperitoneally with liver puncture material from case No F 1734 and killed on March 15, 1927 The spleen of the animal was much enlarged and both liver and spleen contained very large numbers of *Leishmania*

Twelve hamsters were inoculated from this animal and the results are indicated in the following table

Table IV

Number of hamster	Interval between inoculation and death	Result of post mortem examination				Percentage of sandflies showing flagellates when fed on hamster
		Size of spleen	Number of parasites in—			
			Liver	Spleen	Bone marrow	
C 1	3½ months	Enlarged	+++	+	++	None
C 2	2 months	Normal	Neg	Neg	Neg	—
C 3	5 months	Enlarged			Neg	None
C 4	0 days	Normal	+	Neg	Neg	
C 5	3½ months	Much enlarged	+	+	+	1 out of 6
C 6	5 weeks	Enlarged	+		Neg	—
C 7	5 months	Much enlarged	++++	+++	+++	None
C 8	5 months	Much enlarged	++			None
C 9	5 months	Slightly enlarged	+	Neg	Neg	None
C 10	3½ months	Enlarged	+++	+++	++++	None
C 11	5 months	Enlarged	++	++	+	None
C 12	5 months	Slightly enlarged	Neg	+	Neg	None

Human Strain D—On July 5, 1926, hamster No 468 was inoculated intraperitoneally with liver puncture material from case No F 1836 and killed on March 15 1927 The spleen of this animal was enlarged and the liver, spleen and bone marrow all contained very large numbers of parasites

Twelve hamsters were inoculated from this animal and the results are indicated in the following table —

Table V

Number of hamster	Interval between inoculation and death	Result of post mortem examination				Percentage of sandflies showing flagellates when fed on hamster
		Size of spleen	Number of parasites in—			
			Liver	Spleen	Bone marrow	
D 1	5 months	Slightly enlarged	+++	+	+++	None
D 2	5 months	Much enlarged	+++	++++	+++	None
D 3	5 months	Slightly enlarged	+	+	Neg	None
D 4	2 months	Enlarged	+	+	Neg	–
D 5	5 months	Enlarged	++++	++++	++++	None
D 6	5 months	Enlarged	++++	++++	+++	None
D 7	5 months	Much enlarged	+	+	+	None
D 8	3½ months	Slightly enlarged	+	Degenerated		None
D 9	5 months	Much enlarged	++	++	++	None
D 10	3½ months	Slightly enlarged	+	+++	+	2 out of 19 (−11)
D 11	5 months	Much enlarged	+++	+++	++++	1 out of 18 (−12)
D 12	5 months	Much enlarged	++	+	++	None

Human Strain E—On June 18 1926, hamster No 430 was inoculated intraperitoneally with liver puncture material from case No F 1733 and killed on March 15, 1927 The spleen of this animal was considerably enlarged, but the liver and spleen contained only small numbers of *Leishmania* and the bone marrow was negative Twelve hamsters were inoculated from this animal with the following results —

Table VI.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen	Number of parasites in —			
			Liver.	Spleen.	Bone-marrow.	
E 1	6 weeks	Slightly enlarged	Neg	Neg	Neg	—
E 2	5 months	Not enlarged	+	+	+	None.
E 3	5 months	Not enlarged	Neg	Neg	Neg	None.
E 4	5 months	Not enlarged	Neg	Neg	Neg	None.
E 5	5 months	Slightly enlarged	+	+	Neg.	None.
E 6	3 months	Slightly enlarged	+	+	+	None.
E 7	5 months	Slightly enlarged	+	++	+	None.
E 8	5 months	Slightly enlarged	Neg	Neg	Neg	None.
E 9	5 months	Enlarged	+	+	Neg	None.
E 10	6 weeks	Normal	Neg	Neg	Neg.	—
E 11	5 months	Not enlarged	Neg	Neg	Neg	None.
E 12	4 months	Slightly enlarged	Neg	Neg	Neg	None.

A comparison of these five strains A to E shows what different degrees of virulence are met with in nature. All five patients were advanced cases of Kala Azar with no obvious differences in the clinical symptoms, yet by sub-inoculation into hamsters very different results were obtained as regards the infectivity of the parasite.

Strains A and B both possess a comparatively high degree of infectivity to hamsters, and also to sandflies fed upon them, for in strain A six out of eight animals were infective to sandflies, and in strain B five out of six. It is noteworthy, moreover, that in strain A a high degree of infectivity to sandflies might be associated with a mild infection in the hamster, as in experiment A 1. Strain C shows a high degree of infectivity in the vertebrate host, for all the hamsters inoculated except one became infected with *Leishmania* in varying degrees of intensity. With the exception of one insect, however, all sandflies fed on these animals remained negative, so in this strain a high degree of virulence in the vertebrate host is accompanied by an almost complete lack of infectivity towards the insect host. Strain D also shows the same high infectivity towards the vertebrate host, and low infectivity to the sandfly.

Strain E is an example of one with a very low degree of infectivity in the hamster, and also in the sandfly, for none of the insects fed on animals infected with this strain showed any development of flagellates.

The presence of such different strains, revealed by the detailed investigation of only five cases of the disease, suggests that in nature a similar, if not greater, range of variation may be expected to occur. The different results obtained by feeding sandflies on patients, as described above (p. 601), also supports this view, and most of the Chinese cases that we have examined would seem to possess a comparatively low degree of infectivity to the insect host.

The occurrence of strains of such varying degrees of virulence doubtless explains the epidemiology of the disease, which, as is well known, is very unequally distributed in the endemic areas.

Other things being equal, when a strain with a high degree of infectivity is present, a large number of cases may be expected to develop in the neighbourhood, but when, as is the general rule in China, the strain has only a low degree of infectivity, very few cases will occur; so one finds occasional villages in which a considerable proportion of the inhabitants are infected, but in the great majority of the towns or villages only three or four cases will be present in each. It is suggestive of different degrees of virulence that in highly infected villages the disease is much more feared, and according to local reports seems to be more rapidly fatal, than in regions where only isolated cases occur.

Transmission Experiments in 1927.

The methods used in 1927 were identical with those employed by the Kala Azar Commission during the previous year and described by Hindle and Patton (1927).

Experiments with Phlebotomus major var. *chinensis.*

(*a*) *Feeding Experiments.*—Twenty-six hamsters were exposed to the bites of sandflies which had fed on infected hamsters, respectively, 3, 4, 5, 6, 7 and 8 days previously. In every case the flies were proved to contain flagellates by subsequent dissection, and in one instance, S 151, these parasites produced infection when inoculated intraperitoneally into another animal. All the hamsters remained negative after intervals of approximately 6 months.

(*b*) *Intracutaneous Inoculation.*—Five hamsters were inoculated intracutaneously with the contents of infected sandflies at various intervals after their infective feeds, but all the animals remained negative.

(*c*) *Intraperitoneal Inoculation.*—A total of 124 hamsters were inoculated intraperitoneally with saline suspensions of the gut contents of one or more sandflies that had fed on an infected animal respectively 2 to 10 days previously, some of the flies having re-fed once or twice. Seven of these animals subsequently became infected with *Leishmania*, and the particulars of these positive experiments are as follows:—

Experiment S 92.—Hamster inoculated intraperitoneally with contents of seven infected sandflies fed 6 days previously on infected hamster 141 and kept at 25° C. Six months later this animal was killed and found to be heavily infected with *Leishmania*. Two other hamsters each inoculated with the contents of a single infected sandfly from the same batch remained uninfected.

Experiment S 104.—Hamster inoculated intraperitoneally with contents of five infected sandflies, fed 6 days previously on infected hamster 331, and kept at 25° C. Six-and-a-half months later the animal was killed and found to be well infected with *Leishmania*. Two other hamsters inoculated with the contents of sandflies from the same batch remained negative, although one of them received the contents of four, and the other of five, infected insects after 5 and 6 days' interval, respectively.

Experiment S 151.—Giant hamster, inoculated intraperitoneally with contents of 12 infected sandflies, fed 7 days previously on infected hamster 382, and refed 4 days previously on a normal animal; one fly was refed a second time the day before it was dissected. The flies were kept at 25° C. Nearly 7 months later the hamster was killed and found to be moderately well infected with *Leishmania*. Four hamsters that had been exposed to the bites of this batch of sandflies remained negative and also three other animals that received inoculations of the contents of infected flies.

Experiment S 167.—Hamster, inoculated intraperitoneally with the contents of one infected sandfly fed 9 days previously on infected hamster 141 and re-fed 4 days later on a normal animal. The flies were kept at 25° C Six months later the hamster was killed and found to have a very slight infection with *Leishmania*. Two other hamsters also inoculated with the contents of infected sandflies from the same batch remained uninfected, and also two other animals exposed to the bites of infected sandflies.

Experiment S 173.—Giant hamster, inoculated intraperitoneally with contents of six infected sandflies fed 6 days previously on infected hamster 141 and kept at 26° C. Six months later this giant hamster was killed and found to be moderately well infected with *Leishmania* Another giant hamster inoculated with the contents of six flies from the same batch, which were dissected the previous day, remained uninfected.

Experiment S 184.—Giant hamster, inoculated intraperitoneally with contents of three infected sandflies fed 6 days previously on infected hamster 738 and kept at 27° C. Six months later this animal was killed and found to be heavily infected with *Leishmania*. Another giant hamster inoculated with the contents of three flies from the same batch 5 days after the infective feed remained negative, and also one exposed to the bites of these flies.

Experiment S 215.—Giant hamster, inoculated intraperitoneally with the contents of one infected sandfly fed 5 days previously on infected hamster 738 and kept at 28° C. The animal was killed nearly 6 months later and found to be heavily infected with *Leishmania*.

Experiments with P. sergenti.

(*a*) *Intracutaneous Inoculation*.—Forty-one hamsters were inoculated intracutaneously with flagellates from the guts of sandflies previously fed at various intervals on infected hamsters. In every case the animals remained negative, although in one instance, when the same dose was inoculated intraperitoneally, the hamster became infected with *Leishmania*.

(*b*) *Intraperitoneal Inoculation*.—Forty-eight hamsters were inoculated intraperitoneally with saline suspensions of the gut contents of one or more sandflies previously fed on infected hamsters at various intervals. Two animals subsequently became infected with *Leishmania* and particulars of these experiments are given below —

Experiment S 15 —Hamster, inoculated intraperitoneally with contents of eight infected sandflies fed 3 days previously on infected hamster No 331 (see above) and kept at 25° C Six and a half months later the hamster was killed and found to be heavily infected with *Leishmania*

Experiment S 43 —Hamster, inoculated with contents of 27 infected sandflies fed 4 days previously on hamster No 331 (see above, p 606) and kept at 25° C Six and a half months later this hamster was killed and found to be very heavily infected with *Leishmania*

Discussion of Transmission Experiments

The results of these experiments with both species of sandflies show that *Leishmania* in an infective state may develop in the alimentary canal of insects which have fed on animals suffering from this disease The negative results of the feeding experiments agree with those obtained last year, but in no degree invalidate the author's view that in China *Phlebotomus major* var *chinensis* is responsible for the transmission of *Leishmania* Experimental difficulties prevented us from exposing hamsters to the bites of large numbers of infected sandflies at the same time, which must often occur in nature when a person may be bitten by a hundred or more sandflies in the course of a single night Moreover the subcutaneous method of inoculation has been shown to be much less liable to produce infection than the intraperitoneal one, and in the case of hamsters inoculated intraperitoneally with the whole contents of varying numbers of infected *P major* only 7 out or 124 or approximately 1 in 18, became infected.

Congenital Transmission

The occurrence of Kala Azar in Chinese infants which, by reason of their age could not possibly have been exposed to the bites of sandflies, shows that other methods of infection must take place occasionally One of the most striking of these cases, for particulars of which I am indebted to Dr Lei of the London Mission Hospital Tientsin, is recorded in detail

The patient, a four months' old Chinese baby was brought by its mother to the out-patient department of this hospital on March 19, 1926 On examination, the spleen was found to be greatly enlarged extending half way across the abdomen, and the child was very anæmic and emaciated The mother stated that she first noticed a lump in the abdomen about two months previously, and the baby was then taken to a local Chinese practitioner who diagnosed the affection as " P'i Chi," or spleen disease " In spite of taking a good deal of Chinese medicines the baby showed no improvement, suffered from diarrhœa and grew thinner In view of the age of the child Dr Lei was doubtful as to its being a case of Kala Azar and therefore made a spleen puncture and found abundant *Leishmania*

The main interest of this case lies in the fact that it could not possibly have been exposed to the bites of sandflies as their season ended approximately two months before the child was born Although the mother showed no obvious signs of disease it is difficult of explanation except on the hypothesis of congenital transmission Low and Cook (1926) recorded a case of Indian Kala Azar in a child born in England and there can be no doubt that in this patient the infection was derived from the mother who was also infected

In the Tientsin case mentioned above the early development of the disease after only eight weeks strongly supports the view that infection took place *in utero* even though the mother was not shown to be infected Dr Marshall Hertig kindly informed me of a similar case at Hsu Chowfu in which the patient a five months old child was successfully treated for Kala Azar at the local mission hospital This infant also from the date of its birth could never have been exposed to the bites of sandflies During 1927 enquiries were made in the neighbourhood of Wei hsien where Kala Azar is endemic and other cases discovered of infants with large spleens that could not have been bitten by sandflies Although we were unable to obtain punctures of these cases the clinical symptoms were characteristic of the disease Moreover the splenic enlargement in children was so well known in the neighbourhood as to have received a special name Nai P i or milk spleen Many of the inhabitants of endemic villages including our assistant Mr I ang I an Chow record having suffered from this splenic enlargement in infancy and state that the disease is often fatal

Although the evidence is incomplete there seems to be no other obvious explanation of this splenomegaly except Kala Azar and one is led to assume that not only may the disease be transmitted congenitally but also a number of cases recover from the infection without treatment Apart from the statements of persons who seem to have had the disease and recovered from it we have met cases of undoubted Kala Azar in which the patients stated that they had suffered from enlarged spleen for many years—in one case as long as 25 years—and it is only a slight step from such a chronic infection to one which is no longer fatal

Experiments with hamsters support the view that a certain number of cases recover without treatment as a small proportion of these animals became negative after having been shown to be infected Attempts to demonstrate congenital transmission in these animals failed, as we were unable to get them to breed in captivity. A dog which was heavily infected with Chinese Kala Azar, the result of inoculation, had a litter of three pups which were stillborn, but the microscopic examination of smears from their organs, and also cultures, were negative.

Summary of Results.

1 *Phlebotomus major* var. *chinensis* is the most favourable species for the development of *Leishmania*, and in this insect the flagellates become attached to the lining of the mid-gut and grow forward until they reach the anterior part of the gut. Invasion of the pharynx usually takes 6 days and under favourable conditions about 25 per cent. of the flies show a proboscis infection.

2. *Phlebotomus sergenti* is an equally favourable host for the early development, but in this species the infection remains confined to the broad posterior regions of the mid-gut, does not beome attached to the lining of the gut, and never invades the proboscis. The infection is dependent on the presence of undigested food material and soon disappears when the gut is empty.

3. Four out of 14 patients infected with Kala Azar gave positive results when sandflies were fed on them, but only about 5 per cent. of the flies became infected.

4. Experiments with a large series of hamsters infected with Chinese Kala Azar show that considerable variation may be observed in the proportion of sandflies that show development of flagellates after feeding on infected animals, ranging from completely negative up to practically 100 per cent.

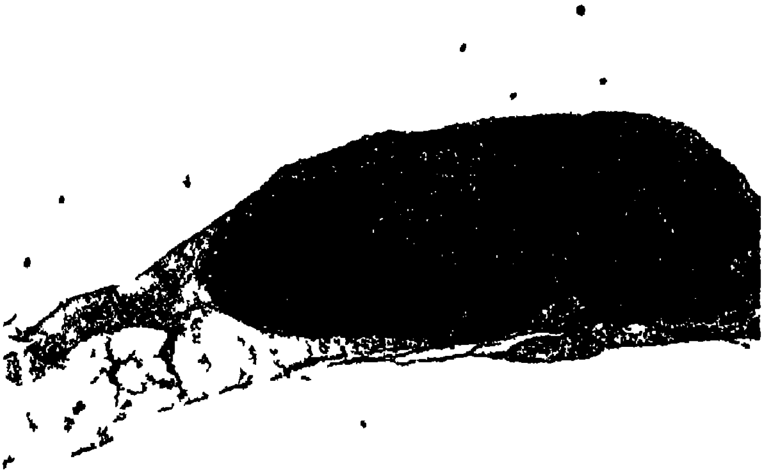
5. In hamsters there is a marked correlation between the number of parasites in the skin and the proportion of flies becoming infected.

6. No correlation could be found between the presence of parasites in the circulating blood and infectivity to sandflies.

7. A study of five human strains in hamsters showed the existence of varying degrees of virulence, ranging from a strain which was completely non-infective to sandflies, up to one which had a high degree of infectivity both to hamsters and to sandflies.

8. Both *P. major* var. *chinensis* and *P. sergenti*, infected with flagellates, gave negative results when fed on normal hamsters, and also when their contents were inoculated into the skin of hamsters. The intraperitoneal inoculation of the contents of one or more infected flies gave positive results in 7 out of 124 experiments with *P. major*, and 2 out of 41 with *P. sergenti*.

9. There is evidence in support of the view that congenital transmission



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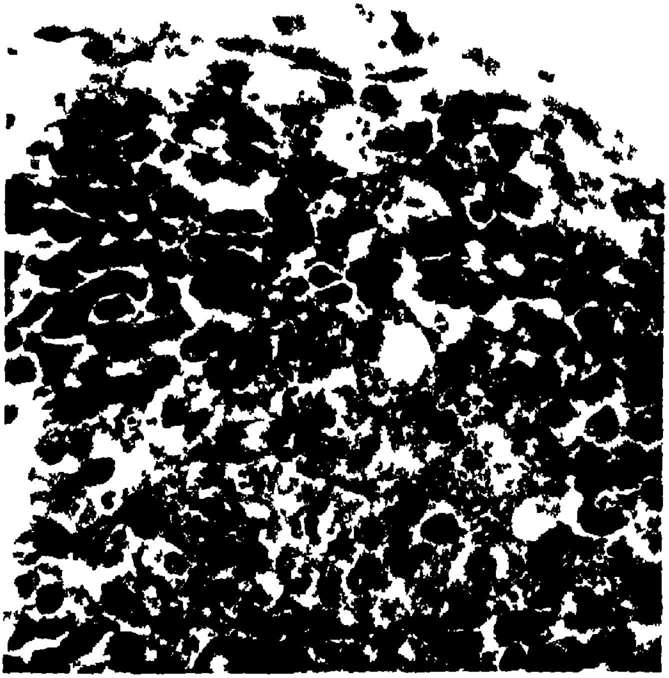

2

The Coefficient of Diffusion of Lactic Acid through Muscle.

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In a recent paper from this laboratory, Stella (1) described experiments relating to the diffusion of inorganic phosphate through muscle. The upper legs of a frog, fresh or in any required stage of fatigue, were suspended at time zero in an excess of Ringer's solution, and at various times t thereafter the phosphate content of the solution was determined. It was assumed that the diffusion obeys the equation

$$P = 2c\sqrt{kt/\pi}$$

where P is the number of milligrams of phosphate diffusing across 1 sq. cm. of the outer surface of the muscles employed, c the initial concentration of the phosphate in milligrams per cubic centimetre, k the coefficient of diffusion (cm.^{2}/min.), and t the time of diffusion in minutes. The mean value of k determined from the observations was 5×10^{-6}, which is far less than that of analogous substances through water; Landolt-Bornstein's Tables give, for example, for oxalic acid $5 \cdot 2 \times 10^{-4}$, for sodium formate 5×10^{-4}, for potassium carbonate $4 \cdot 2 \times 10^{-4}$. The small value of k showed that diffusion of phosphate through the fibres is far slower than in free solution.

This method of determining the speed of diffusion is very convenient in dealing with living tissues. In principle it is only approximate; in practice, however, within certain limits, it yields results of considerable accuracy. Mathematically speaking the formula applies to the case of a semi-infinite solid bounded by the plane $x = 0$; by this is implied that the solid lies to the right of that plane, from $x = 0$ to $x = \infty$, while well-stirred liquid in contact with it lies to the left. At $t = 0$ the concentration in the solid is everywhere c; in the liquid the concentration—owing to the mixing—is always zero. The *rate* of diffusion across the surface at any subsequent time t is then, per unit of area,

$$\text{Rate} = c\sqrt{k/\pi t} \qquad (1)$$

and the *amount* crossing unit area in time t (reckoning from the start) is

$$\text{Amount} = 2c\sqrt{kt/\pi} \qquad (2)$$

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Now, provided that the time t be not too great, any body, of not too irregular shape, can be treated as a semi-infinite solid. It has merely to be so large that in time t the concentration in its inner regions is not appreciably affected by diffusion. For very short times equations (1) and (2) hold for a body of any size and shape; the amount is proportional to the square root of the time. So long as this proportion holds, the constant of the proportion is $2c\sqrt{k/\pi}$; with longer times it ceases to hold, as the concentration within the body changes. If, however, we know experimentally that the total amount diffusing per square centimetre of surface (reckoned from the start), divided by the square root of the time (reckoned from $t = 0$), is the same for times t_1 and t_2 ($t_1 < t_2$), then the constant of the proportion is $2c\sqrt{k/\pi}$. Obtaining c by analysis we can determine k.

It is assumed that c is initially uniform within the tissue. If we are dealing with the case of diffusion from a fatigued muscle any treatment (such as contact with oxygen, or a preliminary washing) which might decrease the concentration of the substance in question in the surface layer must be avoided. Moreover, for strict accuracy, c must be reckoned in units of mass (*e.g.*, milligrams) *per cubic centimetre*, and not per gram.

In the present experiments a pair of frog's legs was skinned and stimulated in nitrogen. It was then plunged rapidly into 20 c.c. of oxygen-free* bicarbonate-buffered Ringer's solution and allowed to remain there for a measured time t. The solution was then removed for analysis and the muscles placed in a fresh 20 c.c. sample. They were then left for a further time $(t_2 - t_1)$ in the solution, which was again removed for analysis. Always two, and sometimes three, such successive samples were used. The concentration of lactate in the Ringer's solution rarely exceeded 1 per cent. of that in the muscles. The surface A of the muscles was then determined in square centimetres, by covering them with strips of paper and measuring the total area of the paper. They were then finally analysed for lactic acid in the ordinary way.

Protein-free extract of tissue, or Ringer, prepared by the use of trichloracetic acid, was neutralised and treated with $CuSO_4$ and $Ca(OH)_2$ to remove sugars (van Slyke). The filtrate was estimated on the principle of Clausen (2) (oxidation with dilute $KMnO_4$) but without aeration (Meyerhof (3)), the oxidation being katalysed by $MnSO_4$ (Shaffer and Cotonio (4)). Quantities of 0·2 to 1·5 mgr. of lactic acid were usually estimated, the technique having, in this range, an efficiency of 97½ per cent., as judged by the recovery of added lactate.

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* Cyanide may not be used to prevent oxidation, because in the subsequent estimation the cyanide behaves similarly to lactic acid (giving rise probably to formaldehyde) causing large errors which cannot be allowed for accurately.

If P_1 be the amount diffusing out in time t_1, and P_2 the amount in time t_2, the quantities $P_1/\sqrt{t_1}$ and $P_2/\sqrt{t_2}$ should be equal within the limits of experimental error. If the muscles be of volume V, and the amount of lactic acid finally found in them Q, the initial concentration c must have been $(Q + P_3)/V$. The value of k may then be calculated.

The data of a typical experiment may be of interest. In this case three times, t_1, t_2 and t_3 were employed, and the corresponding amounts P_1, P_2 and P_3 diffusing out determined. The amount of lactate at the beginning (calculated as lactic acid) was 4·9 mgrs. in 4·7 c.c. of muscles (volume calculated from weight and specific gravity); hence c = 1·05. A was 17 sq. cm. The following results were obtained:—

t_1 12·5 mins. t_2 27·5. t_3 49.
$P_1 = 0·59$ mgrs. $P_2 = 0·86$. P_3 1·18.
$P_1/\sqrt{t_1}$ 0·167. $P_2/\sqrt{t_2}$ 0·164 $P_3/\sqrt{t_3}$ 0·169.

Taking the mean value of $P/\sqrt{t} = 0·167$, and substituting in equation (2) we find

$$k = 6·9 \times 10^{-5}$$

The following table contains the results of all experiments regarded, on grounds other than the value of k finally obtained, as being reliable.

The results for the stimulated muscles are shown graphically in fig. 1. It

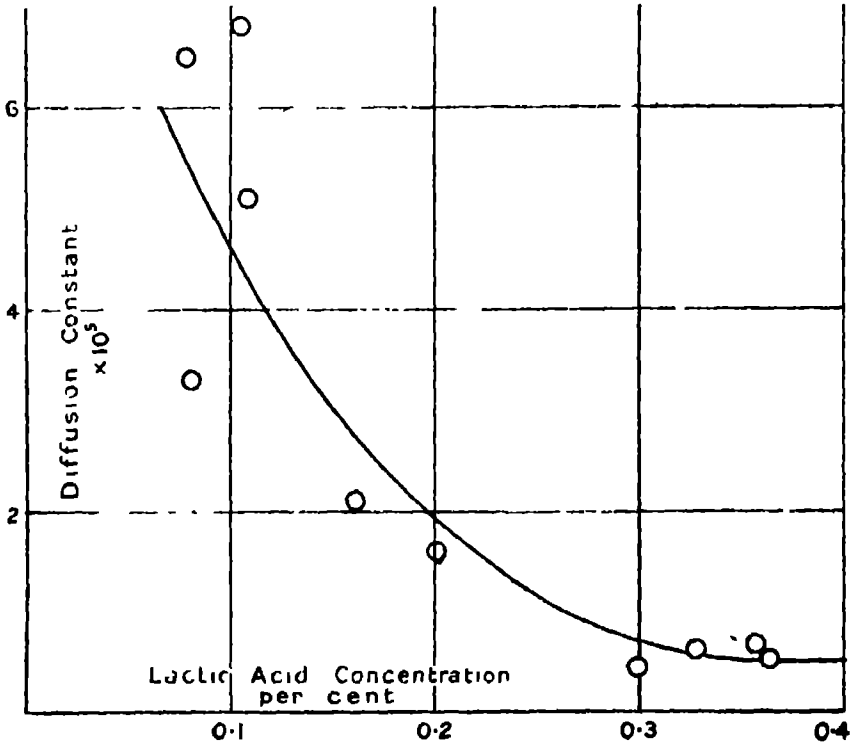

FIG. 1.

FIG. 1.—The diffusion "constant" of lactic acid out of living frog's muscle as a function of the initial lactic acid concentration of the muscle.

Table I.—Values of k, the Coefficient of Diffusion of Lactic Acid through Muscle.

S represents stimulated muscles; R_1 represents muscles dead at 37° C. but not yet rigid; R_2 muscles dead and rigid at 37° C.; R_3 muscles dead, rigid and kept at 37° C. for 3 hours; R_4 muscles dead and rigid at 45° C. In the first part of the table the experiments are arranged in the order of their initial lactic acid concentrations, c.

Date.		c (milligrams) per cubic centimetre	Amount of lactate diffusing (milligrams), and time (minutes).	Observed values of k	Mean value of k
30.3.28	S	0·078	0·26 (7½′). 0·52 (30′)	$6·5 \times 10^{-6}$: $6·5 \times 10^{-6}$	$6·5 \times 10^{-6}$
19.4.28	S	0·081	0·345 (10′) : 0·74 (42½′)	$3·2 \times 10^{-6}$: $3·5 \times 10^{-6}$	$3·3 \times 10^{-6}$
1.12.27	S	0·105	0·59 (12½′) : 0·86 (27½′) : 1·18 (49′)	$6·9 \times 10^{-6}$: $6·6 \times 10^{-6}$: $7·0 \times 10^{-6}$	$6·8 \times 10^{-6}$
30.3.28	S	0·108	0·40 (7½′). 0·82 (30′)	$4·9 \times 10^{-6}$: $5·2 \times 10^{-6}$	$5·1 \times 10^{-6}$
13.4.28	S	0·161	0·34 (7½′) : 0·72 (30′)	$2·0 \times 10^{-6}$: $2·2 \times 10^{-6}$	$2·1 \times 10^{-6}$
13.4.28	S	0·201	0·36 (7½′) : 0·92 (30′)	$1·2 \times 10^{-6}$: $2·0 \times 10^{-6}$	$1·6 \times 10^{-6}$
24.11.27	S	0·299	0·31 (15½′); 0·90 (35′) : 1·19 (52½′)	$3·3 \times 10^{-6}$: $4·6 \times 10^{-6}$: $5·4 \times 10^{-6}$	$4·4 \times 10^{-6}$
14.12.27	S	0·328	0·37 (4′). 0·69 (16′) : 1·11 (37½′)	$6·5 \times 10^{-6}$: $5·7 \times 10^{-6}$: $6·2 \times 10^{-6}$	$6·1 \times 10^{-6}$
19.4.28	S	0·357	0·47 (7½′) : 1·25 (30′)	$4·9 \times 10^{-6}$: $8·7 \times 10^{-6}$	$6·8 \times 10^{-6}$
12.12.27	S	0·364	0·63 (15′) : 1·0 (31′) : 1·27 (42′)	$4·3 \times 10^{-6}$: $5·2 \times 10^{-6}$: $6·2 \times 10^{-6}$	$5·2 \times 10^{-6}$
5.3.28	R_1	0·588	1·49 (7½′) : 2·92 (30′)	$1·75 \times 10^{-5}$: $1·7 \times 10^{-5}$	$1·7 \times 10^{-5}$
5.3.28	R_1	0·562	1·4 (7½′) : 2·65 (30′)	$1·65 \times 10^{-5}$: $1·5 \times 10^{-5}$	$1·6 \times 10^{-5}$
14.4.28	R_2	0·541	0·8 (7½′) : 1·83 (30′)	$1·65 \times 10^{-5}$: $2·15 \times 10^{-5}$	$1·9 \times 10^{-5}$
14.4.28	R_2	0·520	1·1 (7½′) : 2·45 (30′)	$1·75 \times 10^{-5}$: $2·15 \times 10^{-5}$	$1·95 \times 10^{-5}$
19.4.28	R_3	0·614	3·47 (7½′) : 6·29 (30′)	$12·1 \times 10^{-5}$: 10×10^{-5}	$11·1 \times 10^{-5}$
16.1.28	R_4	0·525	2·26 (8′) : 4·5 (32′)	$6·3 \times 10^{-5}$: $6·3 \times 10^{-5}$	$6·3 \times 10^{-5}$
16.1.28	R_4	0·499	3·25 (8′) : 6·24 (32′)	$10·7 \times 10^{-5}$: $9·9 \times 10^{-5}$	$10·3 \times 10^{-5}$

is clear that the coefficient of diffusion is far from independent of the state of fatigue of the muscle. At high concentrations of lactic acid k approaches a constant value of 5×10^{-6}; at low concentrations it is much larger, being (in the neighbourhood of 0·1 per cent. of lactic acid) about 10 times as great. This difference, however, is not due simply to the concentration of lactic acid; a muscle after heat rigor or incubation, containing considerably more lactic acid, shows not a lower but a higher diffusion constant.

It seems likely that the large change in diffusion constant with fatigue is due to an alteration in the lymph-interspaces of the tissue. In fatigue the osmotic pressure of the inside of the muscle cells rises, and the fluid of the lymph spaces is taken up by a swelling of the cells. If this be so, increasing degree of fatigue should cause a gradual fall of the diffusion constant to the lower value characteristic of the fibres when closely packed. On this view the fall of k with increasing concentration of lactic acid would be due to a diminution in the relative volume of the lymph spaces, and the variability of the results, not chiefly to experimental error (which is comparatively small), but to variation in the fraction of the muscle occupied by lymph spaces between the fibres.

The diffusion constant of lactic acid through the lymph must presumably be in the neighbourhood of $6{\cdot}6 \times 10^{-4}$, the value obtaining (see below) in an agar jelly. The value found for a fatigued muscle (about 5×10^{-6}) is characteristic, on this hypothesis, of diffusion through the fibres themselves. A tissue containing x per cent. of interspaces distributed at random, and $(100 - x)$ per cent. of fibres, would have, therefore, for gross diffusion, a k of $1/100\,[x \times 6{\cdot}6 \times 10^{-4} + (100 - x) \times 5 \times 10^{-6}] = 10^{-6} \times [5 + 6{\cdot}55x]$. Equating this to 6×10^{-5}, *i.e.*, the value (fig. 1) for 0·08 per cent. lactic acid, we find $x = 8$. Thus interspaces occupying about 8 per cent. of the volume of the muscle would give the value of k found in the case of comparatively fresh muscles. This is a reasonable estimate of the interspaces.

When the integrity of the muscle fibres is partially destroyed by heat rigor we should expect to find, as we do find, a diminished resistance to the diffusion of lactic acid. In spite of the high osmotic pressure due to lactic acid formed, k in the three extreme experiments at the bottom of Table I averages considerably more than in the observations on the unfatigued muscles at the top. Presumably the membranes normally hindering diffusion are no longer intact in such cases, and the muscle is tending, in respect of diffusion, to the state of a membraneless jelly.

The low diffusion constants of lactic acid and inorganic phosphate through living muscle are in striking contrast with the high diffusion constant of oxygen. According to Krogh (5) the diffusion constant of oxygen, at 15° C., is $1 \cdot 33 \times 10^{-5}$. This, however, is expressed in an unusual way, namely, for a *gradient of partial pressure* of 1 atmosphere per centimetre, in place of a *gradient of concentration* of 1 c.c. per cubic centimetre per centimetre. The solubility of oxygen at 15° C. being 0·034, the former represents a concentration gradient of 0·034 c.c. per cubic centimetre per centimetre, or only 0·034 times the latter. Thus, expressed in the ordinary way, the diffusion constant of oxygen at 15° C. is 1/0·034 times as great as Krogh's value; it is 4×10^{-4}. This is of the same order of quantities as the coefficient of diffusion of most ordinary substances in free solution. Presumably, therefore, oxygen (perhaps because it is electrically uncharged) travels through the membranes of the living cell as fast, or nearly as fast, as in free solution.

In order to test, by a method analogous to the one employed above, the free diffusion of certain substances important in muscular metabolism, the following set of experiments was performed. An agar jelly was prepared containing creatine 410 mgrs. per 100 c.c., lactate 343 mgrs. per 100 c.c., and phosphate 97½ mgrs. P per 100 c.c., pH about 7·5. This was poured into five tubes, two of them wide with a cross-section of about 4 sq. cm., three of them narrow with a cross-section of about 2 sq. cm. When the jelly had set, water was poured into the tubes at time $t = 0$ and kept agitated, and changed at various times to allow analysis for creatine, lactic acid and phosphate.* The amount of water was such that the concentration of the diffusing substance never rose above 2 per cent. of that in the jelly. The results are shown in fig. 2 where the number of milligrams which diffused out is plotted as a function of the square root of the time. In each case a good straight line results, showing that the use of equation (2) is justified. It will be noticed that the values for the small tubes are very slightly above those for the large. This is due, no doubt, to the fact that the curved rim of the jelly, due to capillarity, where it was in contact with the tube, caused a relatively greater increase of surface in the small tube than in the large. The mean values of k, calculated from the lines in the figure, are

Creatine	$5 \cdot 9 \times 10^{-4}$
Sodium lactate	$6 \cdot 6 \times 10^{-4}$
Sodium phosphate	$5 \cdot 6 \times 10^{-4}$

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* Phosphate was estimated by the method of Briggs (6) as modified by Robison and Martland (7); creatine by the micro-method of Folin (8). Pure creatine was used as the standard of reference.

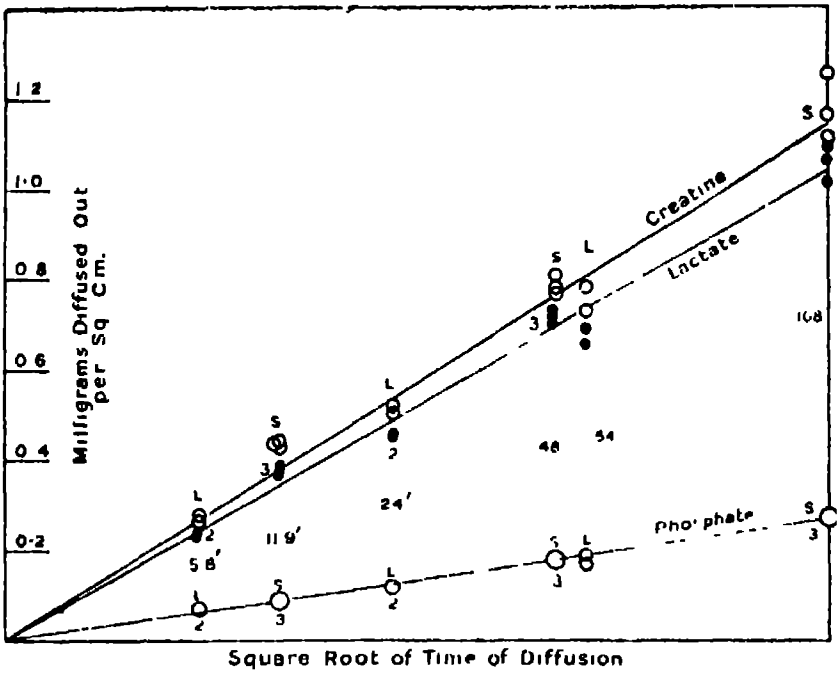

FIG. 2.

FIG. 2.—"Model" experiment with agar jelly containing creatine, sodium lactate and sodium phosphate at approximately the same concentrations as in fatigued muscle. Milligrams of each substance diffusing out, per square centimetre of surface, as a function of the square root of the time of diffusion S denotes small tube. L denotes large tube. Hollow circles denote creatine and phosphate, full circles denote lactate. Time of diffusion for each group shown in minutes thus, 48'. A figure by the side of a plotted point indicates the number of separate observations, in case these are so close as to be indistinguishable in the diagram. Note that the amount diffusing is proportional to the square root of the time, and that the large tube gives points slightly lower than the small, owing to a greater influence of capillarity on the form adopted by the jelly in the tube. For calculated diffusion constants, etc., see text.

These values are of the same order of size as those of oxalic acid, sodium formate and potassium carbonate, referred to at the beginning of this paper; for lactate and phosphate, however, and almost certainly for creatine though we have not examined it quantitatively in muscle, the values are far greater than for the same substances diffusing through a living muscle.

In free solution sodium phosphate and sodium lactate diffuse at practically the same rate. In muscle, resting or fatigued, Stella's value of k for phosphate, viz., 5×10^{-4}, is the same as ours for lactate diffusing through fatigued muscle; our value for lactate in muscle only slightly fatigued is much higher. This suggests that in his experiments the fibres were closely packed in both cases, as we have supposed in the case of the fatigued muscles only. The inconsistency may be due to a difference in the conditions of the two sets of experiments. In ours (see Table I) diffusion was allowed only for a short time, averaging about

20 minutes; in his for a long time, 6 hours. It is well known that prolonged immersion of a muscle in Ringer's solution tends to cause it to swell, a process which probably occurs in the fibres themselves, maybe with an obliteration of the interspaces. If this be so, Stella's value in either case would correspond to diffusion through the fibres tightly packed together and we should expect it to be the same as ours for fatigued muscle.

For muscles in rigor, Stella found a value of k only slightly greater than for live ones, whereas ours is much greater. His rigor, however, was induced by prolonged anaerobic survival at a low temperature, ours rapidly by heat. The latter treatment may well cause a greater breaking up of the membranes of the tissue than the former, with a greater rise in the diffusion constant.

Summary.

1. If an object of any shape containing a soluble diffusible substance in uniform concentration c is brought suddenly in contact with a large quantity of well-stirred solvent, not containing that substance, then the amount which diffuses out bears to the time a relation which, for short times, is

$$\text{amount per square centimetre of surface} = 2c\sqrt{kt/\pi},$$

where k is the diffusion constant and t is the time. The range within which the formula is valid is discussed.

2. This equation may be used to determine the coefficient of diffusion of lactic acid through muscle. The upper legs of a frog, after stimulation, are placed in well-stirred oxygen-free Ringer's solution, and the amount of lactic acid diffusing out in various times is measured. The initial concentration in the muscles, and the surface area, are then determined. So long as the ratio, amount diffused $\div \sqrt{t}$, is constant the above formula is valid, and the value of the ratio allows us to calculate the coefficient of diffusion.

3. In a live muscle with low initial concentrations of lactic acid the value of k is about 6×10^{-5}; with higher concentrations k is diminished, approximately to 5×10^{-6} in extreme fatigue. This diminution is not due to the concentration of lactic acid alone, since muscles after heat-rigor—containing considerably more lactic acid—show a value twice as great as that obtained for relatively unfatigued muscles. It is suggested that the bulk diffusion in a muscle is made up of two factors: (*a*) diffusion through the lymph interspaces, which is as rapid as in free solution, with a constant of about $6 \cdot 6 \times 10^{-4}$; and (*b*) diffusion through the living fibres, which is much slower, with a constant of about 5×10^{-6}. On this view when a muscle is relatively unfatigued the lymph spaces occupy about 8 per cent. of its volume. As the osmotic pressure inside the fibres rises in fatigue the lymph is absorbed and the diffusion constant gradually diminishes to the value characteristic of the fibres closely packed. In the dead muscle, after heat rigor, the

membranes of the fibres are no longer intact, and the diffusion constant tends to rise towards that through free solution.

The expenses of this research have been borne by a grant from the Medical Research Council to P. Eggleton.

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OBITUARY NOTICES

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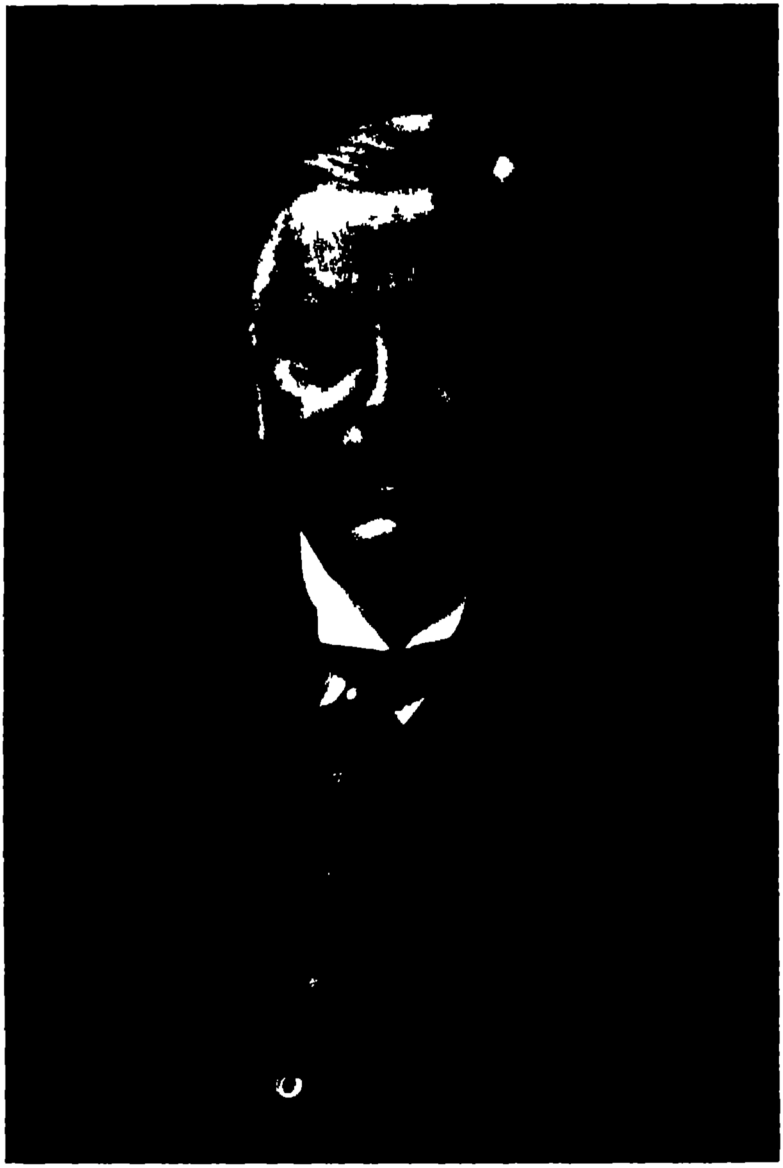


A. E. Shipley

SIR ARTHUR EVERETT SHIPLEY 1861–1927

Sir Arthur Shipley was the second son of Alexander Shipley of the Hall, Datchet, who died in 1896. He was born at Walton on Thames on March 10, 1861, and died at the Master's Lodge of Christ's College, Cambridge, on September 22, 1927. His health had for some time been failing, but it was hoped that a visit to Trinidad, undertaken at the beginning of 1927, might re-establish it. This hope was not realised, and after his return early in March, his condition continued to give his friends anxiety. This was accentuated during Easter week by an attack which for some forty-eight hours seemed likely to prove fatal. He made an unexpected and really wonderful recovery, and for a considerable part of May and June was able to lead an active life, attending to business and taking daily walks both in the College grounds and in the town. The improvement did not continue, however, and although he was able to spend three weeks at Folkestone towards the end of July he was going downhill with fluctuations throughout the summer. The final illness began to show itself towards the end of August. Before his death he was contemplating a short account of the influence of Biological Science on the spread of Colonisation to have been published in the Cambridge History of the British Empire, of which Dr J. Holland Rose is one of the editors, but this essay does not appear to have been begun.

Arthur Shipley was one of several children, of whom two sisters survive—Mrs A. Hutchinson, wife of the present Professor of Mineralogy at Cambridge and Master of Pembroke College, and another who married Dr H. M. Stewart, a well-known medical practitioner in Dulwich and Senior Surgeon of the South Eastern Hospital for Children. His elder brother Sir William Shipley (died 1922), was three times Mayor of Windsor, and his younger brother Reginald (died 1924) went to the Boer War as a captain of the City Imperial Volunteers, served as colonel in the Great War, and received the honour of C.M.G.

In childhood Shipley was markedly delicate, having commenced life as an abnormally small infant. He developed rather late, and is said to have taken no real interest in learning until he entered University College School, London, in 1877. Like many other naturalists he commenced his scientific career as a medical student, and spent a year at St Bartholomew's Hospital. A year later (1880) he entered Christ's College, Cambridge, in the Michaelmas Term. He obtained a First Class in both parts of the Natural Sciences Tripos, Part I in 1882 and Part II in 1884, having been engaged for several months between these two examinations in research work at the Zoological Station at Naples, a place for which he retained a strong affection. He was elected to a Fellowship at Christ's in 1887, and became Master of the College in 1910. In this capacity he served as Vice-Chancellor of the University from 1917 to 1919. He had been appointed Demonstrator of Comparative Anatomy in the University in 1885, Lecturer on Advanced Morphology of the Invertebrata in 1894, and Reader in Zoology in 1908; and for many years from 1891 he had been Secretary to the Museums and Lecture Rooms Syndicate, an important position which made him practically the business manager of the numerous Museums, Laboratories, and other buildings which came under the control of that body. From the time of his entry as a freshman in 1880 till his death he resided in Cambridge, but he travelled extensively and had probably lost count of the number of times he had crossed the Atlantic, principally in visits to the United States, where he had many close personal friends. He was elected into the Royal Society in 1904, serving on its Council from 1909 to 1911, and he was a member of many other learned societies. He was Chairman of the Council of the Marine Biological Association, Treasurer of the Research Defence Society, and a member of the Central Medical War Committee and of the Managing Committee of the Imperial Bureau of Entomology. His scientific distinction, principally as a parasitologist, was recognised by his election as Foreign Member of the American Association of Economic Entomologists and of the Helminthological Society of Washington, and Honorary Member of the Société Royale Zoologique et Malacologique de Belgique and of the Yorkshire Philosophical Society. He was a Trustee of the Hunterian, Tancred, and Beit Foundations, a member of the Royal Commissions on the Civil Service, Trinity College, Dublin, and the Importation of Store Cattle; and of the Departmental Enquiry into Grouse Disease. He received the Honorary Degrees of D.Sc. Princeton, LL.D. Michigan, and M.Sc. Drexel Institute, Philadelphia. His public services were recognised in this country when he was selected for the honour of G.B.E. in 1920.

It follows from this recital that Shipley's career was a many-sided one. He was in the first instance a competent zoologist and a successful researcher and teacher. He had great gifts as a popular writer, who could expound the results of Biological research in an intelligible form, made more attractive by numerous touches of effective humour. From early days he realised the immense importance of Biological knowledge in its practical bearings on Agriculture, health and disease; and a large proportion of his public work was devoted to the application of this knowledge. He did notable service to the country during the Great War.

Shipley and I came up to Cambridge at the same time, in October, 1880. We were the companions of specially brilliant students, many of whom were destined to achieve marked distinction. Among these may be mentioned Adami, Bateson, Chree, Fitzpatrick, J. R. Green, Harker, Head, Sherrington, Threlfall, and D'Arcy Thompson, besides others, such as Adam, Sorley and

Whitehead, in subjects outside the range of the Natural Sciences Tripos. The stimulating effect of this personal environment was increased by the enthusiasm of our teachers, who successfully inculcated in us the belief that knowledge is good for its own sake. The study of Science had quite recently begun to make great strides in Cambridge, although we were not contemporary with the commencement of the modern movement, largely due to Adam Sedgwick and Henslow. The list of University Professors and others working for science was, however, a notable one. Liveing, Humphry, and Michael Foster had been mainly instrumental in establishing the claims of natural science to an honoured place in the University. Lord Rayleigh was Cavendish Professor, and Zoology was represented by Alfred Newton, J. W. Clark, F. M. Balfour, and Adam Sedgwick the younger. Vines, a Fellow of Shipley's College, was engaged in teaching Botany on modern lines. Although I cannot speak too highly of the friendship and scientific assistance we received from Newton and Clark, the influence of Frank Balfour must be mentioned as the outstanding zoological feature of our student days. The effect produced in Cambridge by the news of his death in an Alpine accident on Mont Blanc, in July, 1882, may be estimated by the records Shipley has left in two of his books ('J. W. Clark,' p. 136 and 'Cambridge Cameos,' p. 162). Balfour's personal qualities had endeared him to his pupils in a way few teachers are beloved. His great work on Comparative Embryology had just been published; and Shipley, like others of his pupils, early commenced research work in this field. Before taking Part II of his Tripos he had produced an interesting paper on the structure and development of the Brachiopoda ('Mitth. Zool. Stat. Neapel,' vol. 4, p. 494), and in January, 1887, he published a valuable account of the development of the River Lamprey ('Quart. J. Micr. Sci.,' vol. 27, p. 325). The study of morphology had recently received a special stimulus by the invention, in the Cambridge Laboratory, of a new method of cutting and mounting serial microscopic sections. By means of the automatic microtome of Caldwell and Threlfall, the first instrument of its kind, it became easy to cut sections in continuous ribbons, and to fasten them to slides in such a way that every section occupied its proper place in the series. The study of embryology and anatomy, particularly of many of the groups of Invertebrates, was immensely facilitated by this simple device, an entirely new departure in methods which was fully utilised by Shipley and his contemporaries. He has himself expressed surprise ('Cambridge Cameos,' pp. 143, 162) that embryology and morphology constituted so large a proportion of the research carried on at the end of the last century under the special influence of Darwin's teaching, itself the result of study along widely different lines.

Shipley did not, however, follow up his earlier interests, except by writing articles on Brachiopoda in 'The Cambridge Natural History' (1895) and the

'Encyclopædia Britannica' (1902) He soon turned his attention to the Gephyrea, on which he published his first important memoir in 1890 ("On *Phymosoma varians*," 'Quart J Micr Sci,' vol 31, p 1) This was followed by several other substantial papers on the same group, treated partly on morphological and partly on systematic lines, including descriptions of the species collected by various expeditions The publication of the ten volumes of 'The Cambridge Natural History' (1895–1909), of which he was joint editor, occupied much of his time for some years, but he was meanwhile establishing his reputation as an authority on parasitic worms, on which he published at least forty to fifty papers The consideration of the numerous practical questions arising from the investigation of parasites was no doubt largely instrumental in leading him to a more general study of the economic aspects of Zoology As early as 1887, however he had been sent by the Colonial Office on a mission to investigate a plant disease in the Bermudas, and in 1889 he had published a note on beetles destructive to rice crops in Burma As years passed, he became increasingly interested in the practical applications of Biology, a subject which remained as his most enduring interest He performed a valuable service by participating in original work of this nature and by emphasising, in popular writings, the enormous importance of a knowledge of Biology to mankind Although not by nature or inclination a systematic Entomologist, much of his later work was concerned with Insects and Arachnids During the War, for instance, he published two very attractively written booklets, mainly on these groups, 'The Minor Horrors of War' (1915) and 'More Minor Horrors' (1916) which were widely read, and forcibly brought home to those who were not Zoologists the lesson that a study of Insects is essential to success in war as in peace He was consulted on the establishment, by the Colonial Office of the Imperial Bureau of Entomology, originally founded, under another name, in 1909, with Lord Cromer as its first Chairman, and he served as a member of the Honorary Committee of Management from the beginning until his death At a later period Lord Milner asked his advice with regard to the foundation of a College of Agriculture in the Tropics, and shortly afterwards (in 1919) he appointed a Tropical Agricultural College Committee, with Shipley as its Chairman The project took definite shape in 1921, when the West Indian College of Tropical Agriculture was established, with a Governing Body of which Shipley was Chairman He continued to hold this position until his death, the College having meanwhile become the Imperial College of Tropical Agriculture In 1924 he visited Trinidad, where the College has been erected, to attend the ceremony of laying the foundation stone, and in the course of this journey he went also to Jamaica, New York, and New Orleans in the interests of the College. He re visited Trinidad early in 1927, but, as explained above, the voyage was not successful in improving the condition of his health, which had become unsatisfactory. The value of his work for the College is indicated by a Resolution of the Governing Body which was passed immediately after his death, recording their deep sense of the irreparable public and personal loss sustained by the College, the Governors and the Staff.

Shipley was at all times keenly interested in such subjects as Malaria and Yellow Fever, which are carried by Dipterous Insects, and the Hook worm disease of miners, due to a Nematode parasite: each of these diseases being responsible for an enormous toll of human life and for an almost equally important diminution of human vigour and efficiency.

In 1893 Shipley produced his 'Zoology of the Invertebrata,' a text-book which has been largely used by students; and he collaborated with Prof. E. W. MacBride in writing another text-book ('Zoology,' 1901), a work which has had an equally successful career. He had previously been associated with Dr. Schonland and Prof. Poulton in translating and issuing an English edition of Weismann's researches entitled 'Essays upon Heredity,' a book which greatly assisted in familiarising English readers with Weismann's results. He was editor of the Pitt Press Natural Science Biological Series, and, for a time, of the 'Fauna of British India' series, and co-editor of 'Parasitology' and of the 'Journal of Economic Biology.' He was perhaps at his best as a popular exponent of Zoology, in articles he wrote for 'The Times' and other papers and in various books of which the earliest seems to have been 'Pearls and Parasites' (1908). '"J," a Memoir of John Willis Clark' (1913) is a skilfully written account of a singularly attractive personality, containing much interesting information with regard to Cambridge during the nineteenth century including its earlier half, when conditions were very different from those existing at present, together with a valuable record of the growth of the Natural Science School. During the Great War and while Vice-Chancellor of the University, Shipley was a member of the British University Mission which was sent out by the Foreign Office on the invitation of the Council of Defence at Washington. In this capacity he made a very extensive tour in the United States visiting the principal Universities and helping to interest Americans in the British share in the War. An attractive sketch of this visit during which Peace was declared, was published as 'The Voyage of a Vice-Chancellor' (1919). His small book, entitled 'Life' (1923), was published in response to an invitation by the Cambridge University Press "to write a book which would make students of Elementary Biology think." The effort may be described as eminently successful. The book is divided into a series of chapters illustrating the various manifestations of life by well-chosen instances, and it gives much information which is not at the disposal of every professed Biologist. As in his other popular writings, the style is attractive, lightened by well-placed humour which does not detract from the dignity of the subject. It is interesting to note that he closes on a note of optimism, and concludes that "the world is a better, a cleaner, and a kindlier place to live in than it was in the middle of the last century." His 'Cambridge Cameos' (1924) includes a sketch of the early history of Cambridge and much other interesting matter. He records the discovery of the first proclamation of Henry VIII in certain paper taken down from the ceiling of rooms in the Lodge at Christ's, during the repairs and reconstruction which were undertaken on his becoming Master. The essay on 'The Hunting of the Yale' is a characteristic example of his success in dealing with a subject which is partly zoological. The appreciation of E. A. Wilson, who was one of those who lost their lives in Scott's last Antarctic Expedition, brings out not only the charm of the man he commemorates, but also his own capacity to do justice to the good qualities of a friend. In 'Islands' (1924) he describes some of his travels abroad, giving information about the Imperial College of Agriculture at Trinidad and other places he had visited. Among the more interesting records in this book is a description (p. 95) of a visit he paid to Metchnikoff, at Messina, in 1883, the year of the discovery by that great naturalist of the rôle played by phagocytes in health and disease. The personal results of that discovery are recorded in Metchnikoff's own words : "A zoologist until then, I suddenly became a pathologist."

No memoir of Shipley would be complete if it did not emphasise the value of his public work. He served on innumerable Committees, Councils, and Government Commissions, and he was Vice-Chancellor of the University during the two critical concluding years of the War. At this time he worked unceasingly, in addition to his more routine duties, in making the resources of Cambridge available for employment to the advantage of the country. He was concerned with the organisation of hospitals, with making Cambridge a training place for cadets and for staff officers on leave, and he placed his extensive knowledge of parasites at the disposal of the Government. His hospitality to wounded officers was unending, and he had a constant succession of convalescents living with him at the Lodge, sharing his meals and conversing with him during the evenings, when he might well have found reasons for not showing this kindness by pleading the exacting nature of his ordinary duties. He took great interest in the courses of study for Naval Officers which were instituted in Cambridge after the War.

Several other services he performed for the University should also be mentioned. He was specially interested in the Appointments Board, a body which has done valuable work in finding careers for young graduates. It may be worth while to notice also the action he took in arranging for the annual publication of 'The Cambridge Pocket Diary,' which differs from most of its kind by beginning at the end of September, just before the commencement of the Academic Year, ending at the conclusion of the following Calendar Year. This is a most convenient form for those who are concerned with

Universities and learned Societies, but I think I am right in stating that the idea originated in Oxford, shortly before the first Cambridge Diary appeared. Shipley was responsible for selecting, as the motto of the Diary, the apposite lines, taken from J. K. Stephen :—

> "Years die in July and are dead till September :
> By the first of October the New Year's born :
> It's a sturdy infant in mid-December,
> And reaches its prime some April morn ·
> Hot and weary in June, it must perish soon,
> It is working too hard : it will break : but *here*
> Is the Dawn of the Year."

The wide range of Shipley's interests is further illustrated by the fact that in 1917 he presided at a meeting, held in Christ's Lodge, which resulted in the foundation of the Cambridge University Anglo-Spanish Society (now the C.U. Spanish Society) and in his election as Chairman. He specially exerted himself to assist the Society after the establishment of the Readership in Spanish in October, 1919. The Spanish Ambassador, Señor Merry del Val, with his wife, subsequently stayed at the Lodge, on Shipley's invitation, and addressed the students at a most successful meeting. At a later period, the Chilian Minister, Señor Augustín Edwards, visited Cambridge in similar circumstances, and afterwards became a frequent visitor at the Lodge. These visits stimulated interest in the study of Spanish and led to a large increase in membership of the Society, principally among undergraduates. Shipley took the practical method of keeping up this interest by frequently receiving the members at pleasant gatherings in his study.

Shipley had an unusually wide circle of friends among persons in all degrees of society. He had special pleasure in giving small dinner parties, which he considered more conducive to the true intimacy of friendship than the larger and more formal parties of this nature which were at one time general in Cambridge. He entertained regularly on Sundays at luncheons, and he welcomed men of all ranks and ages who chose to visit him at his informal receptions on Sunday evenings. His knowledge of the world rendered his counsel particularly valuable to his College and University. He was respected by all as a man who consistently maintained the highest standard of public and private duty, and in the midst of responsibilities which might well have absorbed all his attention he was invariably ready to give his time to the performance of acts of kindness. May I add that he was the earliest of my Cambridge scientific friends, and that, as his collaborator for many years in matters in which we were closely associated, I had the best opportunities possible of appreciating his ability, his versatility, his industry, and, above all, his unselfish devotion to duty and kindness of heart.

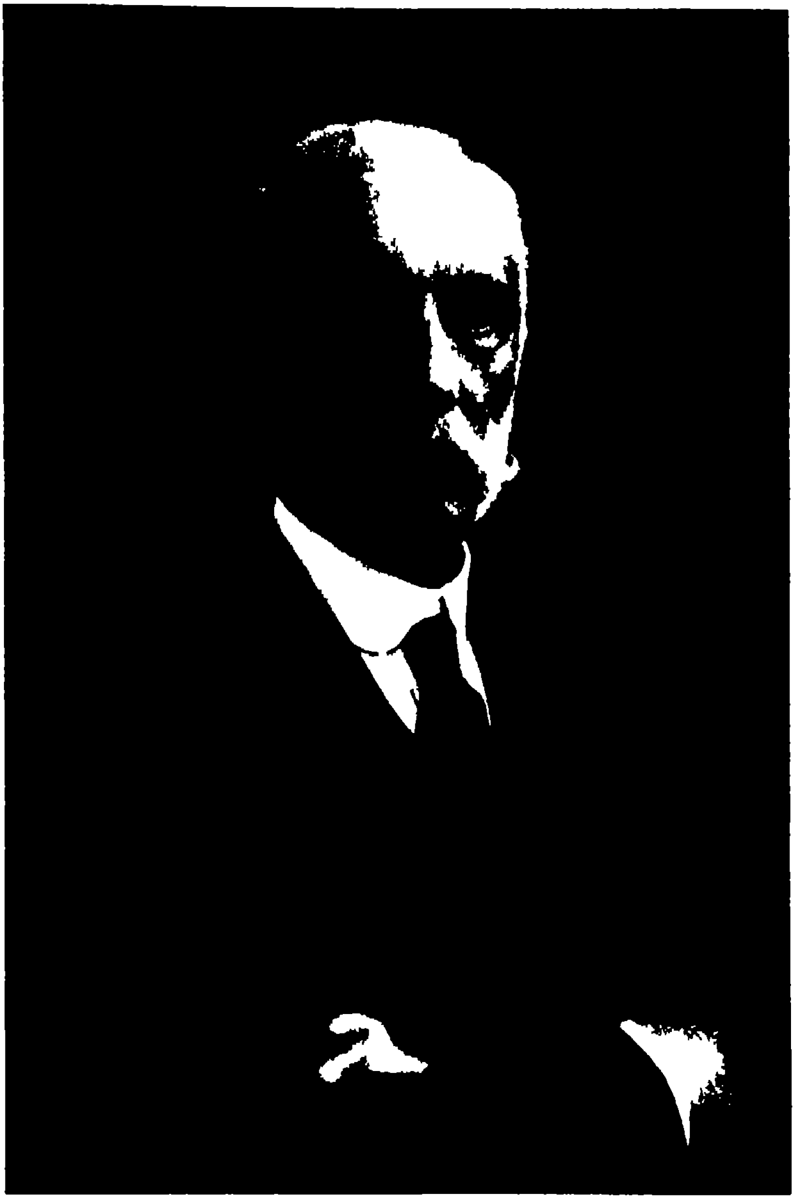
In writing this memoir I have made special use of the notice in ‘ The Times of September 23 I have further to acknowledge the assistance which has been kindly given to me by Sir Arthur s sister Mrs A Hutchinson, by her husband Professor Hutchinson F R S Master of Pembroke and by Mr Algernon Aspinall C M G

S F H

SIR DAVID FERRIER 1843 1928

On March 19 of this year in London where had been his home for nearly sixty years died David Ferrier Elected into the Society in 1876 a pioneer in experimental physiology as applied to the brain he had lived long, constituting in his person a tie between the present generation and a past one which he had himself eminently helped to make of permanent significance to neurology and devoting veteran service to neurology and medicine

Born January 13 1843 he was a younger son of David Ferrier who engaged in business at Woodside near Aberdeen As a boy he had been at the Grammar School and Gymnasium of his native city and at the age of 17 had gained first place in the competition for bursaries at the University there The Faculty he entered was that of Arts In 1863 he took with first class honours in Classics and in Philosophy his M A degree In those subjects he was awarded the Ferguson Scholarship open to graduates of all the four Scottish Universities At Aberdeen University he came under and was indeed a favourite pupil of Alexander Bain the Professor of Logic, author of a well known treatise Body and Mind the first sketch of some chapters of which appeared (in successive numbers of the Fortnightly Review) in 1865 In that year Ferrier went to Edinburgh to study medicine and at Edinburgh by the end of 1868 he graduated M B Then but not for long he acted as assistant to Thomas Laycock the Professor of Practical Medicine, and also engaged, for his circumstances were straitened in coaching students a task which proved very uncongenial to him He forsook it to become assistant to a general practitioner in Suffolk Dr Image, of Bury St Edmunds an accomplished man, bachelier ès lettres and F R C S , and member of a family well known for scholarship and proficiency in natural science one of them classical tutor at Trinity College, Cambridge another a recognised authority on the geology of the district where he lived as rector While with Image, and with the hearty



David Ferrier

co-operation of Image himself, he spent most of his time in research, an investigation into "the comparative anatomy and intimate structure of the Corpora Quadrigemina." This was carried out, as he in later years enjoyed recounting, largely amid the amenities of Image's garden, a place known locally for its beauty, and forming to such a delighted observer of living nature as Ferrier a specially congenial setting for his study. The work was later sent in as an M.D. thesis and awarded a Gold Medal. In after years he again returned to the subject in a research with Dr. Aldren Turner.

In 1870, Ferrier removed to London, and was appointed Lecturer on Physiology at the School of Middlesex Hospital, on the resignation of Burdon Sanderson. He retired from it in the following year to begin his long association with the Medical School of King's College, as Demonstrator of Physiology there. At King's, in 1872, he succeeded Dr. Guy in the Chair of Forensic Medicine, and he held this position until 1889, when he became Professor of Neuropathology, a Chair specially instituted in his honour. In his early years in London he shared a house, since demolished, 53, Somerset Street, with two fellow Edinburgh graduates of his own standing, Lauder Brunton and Milner Fothergill, the former to become a Fellow of the Royal Society a little earlier than himself. Although engaging in practice he from his first arrival embarked on a career of laboratory research. His attention dwelling specially upon the physiology of the nervous system, he had contact with Hughlings-Jackson and with Jackson's views and teaching on the cerebral mechanism of certain forms of convulsions. In March, 1873, when he was paying a visit to his friend and fellow Edinburgh graduate, Dr. (now Sir) James Crichton-Browne, then Director of the West Riding Asylum, Wakefield, conversation turned upon the excitability under galvanism of part of the cerebral surface of the dog as reported from the Continent by Fritsch and Hitzig. There followed, during the course of the spring and summer of 1873, in the laboratory recently founded at the Asylum by its enlightened and progressive Director, who put it at Ferrier's disposal for the purpose and even provided the animal material, the memorable experiments with which Ferrier opened his detailed systematic exploration by faradic stimulation of all parts of the central nervous system in representative types of vertebrate from lowest to highest. A grant of money from the Royal Society helped him to include observations on the brain of the ape, an organ much closer to the human brain than any laid under contribution hitherto—a step historically reminiscent of Galen, if the tradition that Galen executed experiments on apes be in fact correct.

The first publication of Ferrier's experiments dates from 1873 in the Reports of the West Riding Asylum. A fuller account formed the substance of the Croonian Lecture of the Royal Socety delivered by him in February, 1874. He was selected Croonian Lecturer also for the following year, 1875, and as such gave an account of further extensions of his experiments. The activity characteristic of him is evident in the circumstance that the main experimental results were all obtained in the course of a very few years and fully published between 1873 and 1876. As was said of him by Sir John Rose Bradford in the Presidential Address this year to the Royal College of Physicians, of which Ferrier was a Fellow, Ferrier placed the question of cerebral localisation of function on an absolutely certain basis of proved experimental fact. He established the localisation of the 'motor' cortex very much as we now know it. He located it as a region accompanying the Rolandic fissure across the lateral aspect of the hemisphere and extending thence over and upon the hemisphere's median aspect. He pointed out that its extent was greater and its character more detailed in the ape than in any of the types less near to man. He showed that its focal movements were obtainable with such definition and precision that "the experimenter can predict with certainty the result of stimulation of a given region." He went on to determine the effects of destruction of limited portions of the cerebral cortex. He allocated regions specially concerned with vision and with hearing respectively. He showed that the hemiplegias and monoplegias ensuing on injuries within the motor region of the ape were characteristically greater than those produced by similar cerebral lesions in the dog. The symptoms in the ape he stressed as being strikingly akin to those familiar in the clinic. At the International Congress of Medicine of 1881, held in London, he gave a convincing demonstration of his results before a gathering of the neurologists of Europe. Sir Charles Ballance, who was present, relates that there, on the appearance of one of Ferrier's hemiplegic monkeys, the clinician Charcot, of Paris, exclaimed "It is a patient!"—words pregnant as a pronouncement on the case for which Ferrier was arguing, and on the facts for which he was contending, against Goltz, a physiological authority present who, without experience of experiments on the ape, broadly denied to the cerebral cortex all localisation of function.

His Croonian Lectures to the Royal Society were followed by the Gulstonian Lectures to the College of Physicians in 1878, and in the interim by the publication of his "Functions of the Brain" (1876). This book, addressed mainly to physiological and medical circles, interested also a wider audience; it was translated into several languages, and reissued in an enlarged edition in 1886. It and the work it embodied went far to place cerebral localisation in the forefront of neurological interest, not only for the physiologist and physician, but for the anatomist and psychologist and a section even of the general public.

Ferrier did not hesitate, from the operative success attending his experimental venture upon monkeys, to argue that in the human patient, given the essential preliminary of diagnosis as to nature and seat of disease by the physician, surgery could in like manner proceed to operative relief. He asserted that under Lister's surgical procedure (he had Lister as a surgical colleague at King's) the brain offered of itself no prohibitive surgical difficulty. In his Marshall Hall Oration of 1883, after ten years of experience of operating on the brain of animals, he repeated this appeal with no less insistence. Physiological experiment, he declared, although finally and fully demonstrating cerebral localisation, and although showing by its reliable safety upon animals that similar operative success could be achieved on man, had still had no appreciable following from practice in the clinic. The very year of this appeal, MacEwen, of Glasgow, operated for the second time for intracranial disease, and in the year following, namely, 1884, Rickman Godlee, in London, performed his well-known pioneer operation, removing "a cerebral tumour of the size of a walnut the position of which had been correctly localised." A surgeon, himself distinguished in intracranial surgery and versed in the history of its development, has said with penetration and generosity that to Ferrier rather than to the surgeons is primarily due the origination of modern cerebral surgery. Of the results traceable to Ferrier's experiments on the brain of the monkey, one, therefore, has been the practical relief by surgery of patients suffering from certain forms of cerebral tumour and intracranial mischief. That fact lends a touch of irony to the circumstance that in sequel to this beneficent research Ferrier was violently persecuted by anti-vivisectionists, and finally on its account was the object of an action-at-law instituted by them. Their attack he met and defeated, and he showed the main statement in their accusation to be untrue. A further irony in the situation was that the prosecution, carrying from certain quarters malignant opprobrium, brought also general notoriety with immediate increase of his consulting practice. The public reasoned doubtless that an authority persecuted for experimenting on the brain must possess knowledge about it worth consulting.

The work of Ferrier was, above all, a fundamental contribution to the study of the physiology of the brain. As to this, to trace the origins of his enquiry and of the decisive effect it produced, requires regard to some contemporary circumstances. Current scientific opinion, physiological and medical, at that time held that the cerebrum was the "organ of mind" and mind being a unity presented as regards its mode of functioning no detectible spatial differences. The cerebral cortex was thought of as undifferentiated, in so far that a partial destruction or lesion of it wherever situate entailed only a diminution of its functional capacity *en bloc*. Moreover, current doctrine opined that the cerebrum, as was at one time thought of the grey matter of the spinal cord, was irresponsive to all stimuli, electrical or other which physiological experiment had at command. Ferrier, however, at the outset of his student life, even before he began medicine, was early in close touch with one whose outlook, despite metaphysical training, was singularly attached to the scientific ideal of ultimate unity. Bain, as Professor of Logic, kept a detailed model of the human brain upon his lecture table, gave an up-to-date description of the structure of the nervous system in his lectures, and constantly set before his class the union of psychical facts with physiological mechanism His "Mind and Body" opens with the words "Many persons, mocking, ask—What has mind to do with brain-substance, white or grey? Can any facts or laws regarding the spirit of man be gained through a scrutiny of nerve-fibres and nerve cells?" On its last page its summing up runs "The arguments for the two substances have now entirely lost their validity The one substance with two sets of properties, a double faced unity, complies with the exigencies of the case" Psychologist, he expressed the hope "We may possibly unlock the secrets of the structure, may compel the cells and fibres to disclose their meaning and their purpose" From such a *milieu* as this Ferrier's student years passed on to medical observation, and with predilections such as his and a nature which had nothing *laissez faire* about it, we may suppose that the precepts received from Bain contributed an effective factor toward shaping a career which was, however, as to its great results entirely Ferrier's own

Further, when Ferrier moved to London he there found Hughlings-Jackson, and the views Jackson had already brought forward for discussion in 1868 and then put together in the famous "Study of Convulsions" issued by him in 1869 In that analytical study of the clinical picture, since familiar as "Jacksonian epilepsy," Jackson ascribed to the cerebral cortex 'motor centres" which could be stimulated by trauma or by residual effects of trauma, motor centres which were situated in the grey matter of certain cerebral convolutions, "the convolutions surrounding the corpus striatum" These views were against the orthodoxy of the time For Ferrier they proved an impetus to experimental enquiry He prefaces the account of his experiments by stating that they have had for an object the "testing of the theory of Hughlings Jackson that localised and unilateral epilepsies are caused by irritative or 'discharging' lesions of the grey matter of the hemispheres"

Ferrier employed, and recommended, the faradic current as a means to excitation of the cortex Fritsch and Hitzig in observations three years before on the brain of the dog, used as stimulus the closing and opening of a voltaic current On this the method of Ferrier was an important advance, for it enabled the elicitation of sustained and deliberate instead of twitch like and evanescent movement, and did so without inflicting damage on the cerebral tissue It allowed provocation and study of the "march," *i e*, that sequence and spreading of movement which Jackson had noted to be of diagnostic value in the epileptoid convulsion It brought the clonus characteristic of epilepsy under observation as a feature of cerebral "after discharge" Ferrier's choice of stimulation has been followed by all observers since

A conception which Ferrier formed regarding the localisation of function in the cortex held it to be primarily "sensori motor" He conceived his regions which constantly yielded movements in such detailed and systematic array to represent a so-to-say motor executive The regions outside it, injury to which evoked signs of disturbance and defect of sense without motor paralysis, he distinguished, in contradistinction to his "motor region," as "sensory" In short, "motor" and "sensory' formed the ground plan of the scheme of localisation for the cerebral cortex in his pioneer interpretation of it Not "motor" and "sensory," of course with the crudity of the efferent and afferent sides of a simple reflex arc, but yet distinguishably "motor" and "sensory" The difference between this and the old phrenological brain-chart of Gall was bold enough to be indeed startlingly reassuring He made no comment on that, but his first paper to the Royal Society remarked that "a scientific phrenology is regarded as possible" The paper also adds "From the complexity of mental phenomena and the participation in them of both motor and sensory substrata, any system of localisation of mental faculties which does not take both factors into account must be radically false" Bain, speaking of endeavour to reach understanding of an organ so complex as the brain, wrote "If we were to confine ourselves to dissection we should probably attain but small measure of success But another road is open We can begin at the outworks, at the organs of sense and motion, with which the nervous system communicates, we can study their operations during life, we can experimentally vary their circumstances, we can find how they act upon the brain and how the brain reacts upon them"

All this requires no further insistence now Suffice to say that thus it was that Ferrier approached and attacked a problem largely opened by himself, which in the half century since his first dealing with it has proved more and more complex with every renewal of penetration into it New terminology has been introduced and with some advantage, to meet the requirement of fresh shades of discrimination and nuances of interpretation of functional processes inferred and of steps and stages sought to be distinguished by analysis None the less, in essential conformity with Ferrier s original stand point we have to day with regard to the motor region ' the experience of so highly qualified an observer as Dr Gordon Holmes to the effect that "the precentral gyrus" (the 'motor region ' proper) ' has no sensory functions" Further, Ferrier's interpretation which assigned to specific sensory fields the major part of the cerebral surface has alongside it to day those arrived at, along other lines of examination, by comparative anatomy and study of cyto architecture, and by the findings of Pavlov and his collaborators schemes which more even than did Ferrier's original, cover the expanse of the cerebral surface with territories representing the specific receptor systems severally, and find no place for the pure association fields posited by Flechsig Ferrier when breaking the new ground would seem to have formed of it a conception with whose fundamental simplicity there corresponds a fundamental truth. It is, however, more evident now than perhaps it was then that, as regards even animal mind, analytic psychology has not yet got within measurable distance of elements needed for its study in so far as this last involves treatment of mind's relation to a material seat.

A tribute paid to Ferrier's work on the cortex was that it had soon many experimental followers. The next decade saw a flowing tide of research setting toward localisation of this kind or that, in one part or another of the nervous system ; more penetrative modes and aims of analysis came to be little pursued. A localisation vogue reigned for nearly a quarter of a century, and became in due course tedious and relatively infertile. But the importance of the work which ushered it in cannot be forgotten. It is difficult now to think back to a functional neurology which resigned itself to picturing the cerebral cortex as an uncharted sea with for its one accredited feature unanalysed uniformity.

Ferrier's experimenting upon the cerebral cortex covered a period of a decade and more. He contributed also much experimental work on the cerebellum, the corpora quadrigemina, the limb plexuses, etc., researches pursued usually in collaboration. He engaged at the same time continuously in consulting practice. He was successively Assistant Physician, Physician and Consulting Physician to King's College Hospital and to the National Hospital for the Paralysed and Epileptic. Ferrier loved experiment and the following of knowledge by its means. The writer remembers the interest and pleasure afforded to a foreign visitor, Señor Cajal, the histologist, by finding Ferrier in the laboratory, experimenting ; and the impression made by the simple hearty welcome and the frank comradeship which delighted in continuing the experiment and in showing and explaining its each step to the younger colleague from abroad. Even in his latest years Ferrier would attend the laboratory experiments of others and enjoy watching the work in hand, modestly expressing the hope that he was " not in the way." Always a staunch upholder of individual liberty of choice in research, as member of the International Brain Commission set up by the now defunct International Association of Academies, he opposed the proposal for the brains of the eminent to be collected at one centre (Leipsic) for uniform systematic report and permanent museum retention. He always favoured freedom of theme, and he was wholly free from what Bacon has termed " the first distemper of learning, the studying of words and not matter." Indeed, in his lucidity of intent he maybe studied words too little, or rather perhaps accredited them with greater simplicity than in fact is theirs.

Honours came to him. He was awarded the Baly and Moxon medals by the Royal College of Physicians, and besides giving the Gulstonian, gave also the Lamleian and the Croonian Lectures of that College, and the Harveian Oration in 1902. He was laureate of the Academie de Médecine, Paris, in 1878. He received Knighthood in 1911. In 1914 the University of Cambridge conferred on him the Hon. D.Sc. on the occasion of the opening of the new laboratories of Physiology and Psychology.

He disliked controversy, even to the extent of making some sacrifices to avoid it. He nevertheless took a prominent part in several new movements which proved to be memorable for science and medicine in this country. He was one of the small group who in London in the spring of 1876 founded the Physiological Society, and was one of those entrusted with the drafting of its original constitution. He was elected to its Honorary Membership in the January after its Jubilee year 1926. With the 'Journal of Physiology' he was early associated, and his name as a collaborator appeared on its title pages from 1893 until his death. He was one of the founders of 'Brain.' On the suspension of the West Riding Asylum Reports, writes Sir James Crichton-Browne, referring to the changes consequent on his own retirement from Wakefield, it was Ferrier who urged that the work which the Reports had begun, should be in some form continued. It then came to be agreed that a 'Neurological Journal' should be started in London, and thus 'Brain' was launched in 1879, with, for its editorial staff, Sir John Bucknell, Dr. Crichton-Browne, Dr. David Ferrier and Dr. Hughlings-Jackson. Soon attaining world-wide recognition, it passed later to management by the Neurological Society, on the starting in 1886 of that Society, of which again Ferrier was one of the founders, and President in 1894. He was intimately connected with the Research Defence Society from its foundation in 1908; he served it as Honorary Treasurer, often presided at Committee, and had, in spite of declining health of late, persisted in attending its meetings.

Ferrier's association with the Royal Society extended over many years. The Society encouraged his first work by granting a sum which enabled him to extend his observations to apes. It selected him for Croonian Lecturer in the two successive years 1873, 1874, altogether prior to his election to the Fellowship. An experimental biologist reviewing the record of the Croonian Foundation across the perspective given by Transatlantic distance has recently written: "It is literally true that the history of muscle-physiology in the 18th, 19th and 20th centuries has been largely developed at these annual occasions," *i.e.*, the Croonian Lectures of the Society. That remark stands valid not least for the two lectures by Ferrier. The award of a Royal Medal was made to him in 1890. He served on the Council for two successive years, 1886–1888, and again similarly ten years later. He was Chairman of the Sectional Committee for Physiology and Medical Subjects during the years 1909 and 1910. He was a Vice-President of the Society from 1906 to 1908. He continued to attend its meetings until even recently, and at the club dinners held after the meetings was a familiar presence. It is understood that from a fund now raised to memorialise him, a sum is tendered to the Society with the suggestion of founding there a Lecture bearing his name.

Slight and erect in figure, genial and alert in bearing, the burden of years long weighed lightly on him. A reflective quietude of voice and manner tended to veil the underlying energy, no less a part of his nature. Shrewd observer, keenly alive to men and things, and not least to æsthetics and to humour, his talk could exert charm, and owed something to a certain piquant directness and penetrative simplicity enhanced by a remnant of accent from Aberdeen. It was after looking through a new and elaborate atlas of brain-charts that, as the writer remembers well, his comment fell, "We know so much now," accompanied by a quizzical smile, commingling amusement, admiration and despair with just a suspicion of mistrust. More often there was nothing enigmatic about his talk. It would be clear-cut like his teaching, and replete with interest in the scientific events of the time. Walking from the cemetery on the day of Hughlings-Jackson's funeral in 1911 it is told of him that after silent occupation with his own thoughts for some minutes he turned quickly to the younger friend beside him and said: "Well, when I cease to take interest in things it will be time for me to go."

He married, in 1874, Constance, daughter of Mr. C. Waterlow, and to the kindness of Lady Ferrier is owing permission for the reproduction of the portrait prefixed to this obituary notice. There are two children of the marriage, a son and a daughter, of whom the former is the well-known architect, Mr. Claude Ferrier.

C. S. S.

SIR AUBREY STRAHAN, 1852–1928.

Sir Aubrey Strahan, who was Director of the Geological Survey of Great Britain and of the Museum of Practical Geology, London, from 1914 till 1920, died at his residence, Fairfield, Goring, Oxfordshire, on March 4, 1928. Since his retirement from the Geological Survey he had resided mostly in the country, though he had taken an active part in geological affairs, and had served on the Council of the Royal Society from 1921 to 1923, and on that of the Athenæum. His final illness was of short duration, and he is buried in the churchyard of Streatley, near his home.

Strahan was the son of Mr. William Strahan, of Blackmore Hall, near Sidmouth, and was born on April 20, 1852. At the age of 13 he was sent to Eton to the Rev. Herbert Snow's house. In 1870 he left and entered at St. John's College, Cambridge, graduating in 1875, and taking Part II in the Natural Sciences Tripos. At that period St. John's was a very active centre of geological work and teaching, and Strahan decided at an early stage to follow his natural bent and devote himself to geological work. The teaching of Bonney and McKenny Hughes, under the guidance of Sedgwick, apparently

A Strahan

exerted great influence on his mind, and among his associates of those years were several who were destined to achieve distinction in the geological world, such as Teall, Marr, Sollas and Clough.

In May, 1875, Strahan joined the Geological Survey as a temporary assistant geologist, under Sir Andrew Ramsay. His first field work was in south Lancashire and Cheshire, then in Flintshire, where he was engaged, along with De Rance, in revising the mapping of the Carboniferous and Triassic rocks, and in an original survey of the superficial deposits. In connection with this work two papers appeared from his pen in the 'Geological Magazine' and the 'Quarterly Journal of the Geological Society of London' in the year 1879. The map in question, 79 N.W., was published in 1885, accompanied by a short explanatory memoir by Strahan, assisted by R. H. Tiddeman. He continued to work intermittently in this district till 1890, when he published a descriptive account of the Flint, Mold and Ruthin Sheet ('Memoirs of the Geological Survey'), based on his own researches and those of C. E. De Rance. In 1898 he returned to this subject, and published a Supplement to his memoir containing additional information regarding the extension of the coalfield.

In 1886 the mapping of the superficial deposits of the Isle of Wight was taken in hand by Strahan and Clement Reid, and Strahan at the same time revised the Secondary rocks, while Reid revised the Tertiary area. This resulted in the publication of a second edition of Bristow's memoir on the Geology of the Isle of Wight by Reid and Strahan in 1889, along with a new edition of the geological map on the scale of one inch to one mile (published 1888).

Work was next transferred to the adjacent mainland, and Strahan and Reid, in the same manner, revised the old mapping of Dorsetshire and Hampshire. The classic area of Purbeck and Portland was assigned to Strahan, who spent several years on this work. Its completion was marked by the publication of his memoir on the Geology of the Isle of Purbeck and Weymouth in 1898, which remains the standard description of that ground. An interesting feature of the book was a series of photographic views which had been taken by Strahan, who was at that time an enthusiastic photographer. The revised maps of this area were issued in 1895 and 1896.

The exigencies of official programmes of work were responsible for the transfer of Strahan's activities to Westmorland in 1883. In 1894 he again visited the north: the published maps of the Whitehaven coalfield had proved unsatisfactory, and a revision was unavoidable. This was still incomplete, and, as many difficult problems were involved, Strahan was sent north to hasten forward the task, a fact which shows that there was great confidence in his judgment and his executive abilities. The revised map was published in 1895, but no memoir was then prepared and no official description has yet been issued of that coalfield, an omission probably to be explained by the great obscurity of many problems of stratigraphy arising from imperfect knowledge of the underground structure of the Carboniferous rocks.

Meanwhile, much pressure was being brought to bear on the Geological Survey to prepare revised maps of the important coalfields. In South Wales the original survey was carried out between 1837 and 1845, and was very much out of date. Sir Archibald Geikie decided to institute a complete revision of the whole coalfield and Strahan was put in charge of the work. He began his re-survey of that coalfield, in which he remained for the rest of his service as a field geologist, in 1891, but he had no assistance till 1893, when he was joined by Dr. Walcot Gibson. Subsequently the work was accelerated by the allocation of four geologists to that district under the superintendence of Sir A. Strahan. Beginning at Newport in the east, they worked westwards sheet by sheet, and the memoirs were finally completed by the publication of the thirteenth volume in 1921 The geological maps on the scale of six inches and one inch to one mile, and the accompanying explanatory memoirs, were all edited by Strahan, and to most of them he was an important contributor. On their completion they formed the most authoritative account of any British coalfield that had appeared up to that date, and set a standard for future work that was of the highest quality.

On the retirement of Sir Archibald Geikie from the Directorship of the Survey in 1901, Sir Jethro J. H. Teall was appointed his successor, and an extensive re-organisation was carried out, involving an increase of staff and a widening of the scope of the work Sir Aubrey Strahan now became District Geologist in charge of the South Wales District. His abilities as an organiser were acknowledged on all hands, and he took a considerable part in amending the procedure and revising the details of Survey programmes.

A Royal Commission was appointed in 1901 to report on the Coal Supplies of Great Britain. At first Strahan was not a member, and the Survey representative was Sir Jethro Teall; but Strahan's work in South Wales was already recognised as of great importance, and in November, 1903, his name was added to the list of Commissioners. The report ultimately published remains the standard authority on the subject investigated, and much of its value is undoubtedly due to Strahan, who was the most experienced coalfield geologist on the Commission, and whose judgment on geological evidence was seldom at fault. He certainly devoted a vast amount of time and labour to this enquiry, and it not only brought him into prominence as an authority on the subject, but also broadened his information and his outlook on all questions of economic geology in Great Britain.

In 1903 Strahan was elected a Fellow of the Royal Society, and in 1904 he was President of Section C (Geology) of the British Association at Cambridge. He chose for his address the Movements of Post-Carboniferous Age that had folded the rocks of England, giving special prominence to the tectonics of

British coalfields, and of the Secondary and Tertiary strata of the South of England This address showed very clearly his special bent to structural field geology, which was throughout his career his favourite subject of investigation

In 1913 and 1914 Strahan was President of the Geological Society of London In this capacity he delivered two notable addresses, (*a*) on the Configuration of the Palæozoic Platform that underlies the Secondary Rocks of the South and East of England, a question involving the possible occurrence of buried coalfields as yet undiscovered, and (*b*) on Post Glacial Denudation The first of these addresses was on a subject that possessed great attraction for Strahan, and he returned to it in a lecture delivered to the Royal Institution in 1916 The second related to an enquiry on the erosive action of British rivers which was being carried on by a committee of the Royal Geographical Society, of which Strahan was an active member

Sir Jethro Teall retired from the Directorship of the Geological Survey in January, 1914 and Strahan was appointed to succeed him He was thoroughly familiar with the duties, and might have looked forward to an epoch of peaceful development along well established lines, but with the outbreak of war, exacting calls were made upon his services The staff at first was largely depleted by volunteering for the forces, and soon it became evident that the need for geological information was very insistent In every part of the war zones geological maps were required For example a special set of geological maps of Belgium had to be printed for the use of the British Staff, and ultimately geologists were attached to all the principal commands Many special problems also arose in connection with the development of trench warfare At home the demand for the raw materials for British industries, that had hitherto depended mainly on foreign sources of supply, immediately became of great importance Such minerals as wolfram tin ore, barytes, fireclay, siliceous refractories cannel coal oil shale manganese ore, lead and zinc ores, anhydrite, were required either for the manufacture of munitions or for industrial uses Strahan was very much at home in this class of work, and speedily built up an organisation that met the demand satisfactorily Every British industry that was based on mineral supplies received attention, subsequently the information collected during this period formed the nucleus of a series of Special Reports on the Mineral Resources of Great Britain, of which thirty volumes have now been published The lines which he laid down have been followed throughout, and have proved satisfactory This series, together with his memoirs on the South Wales coalfield indicate Strahan's position as one of the greatest contributors to our knowledge of the economic geology of Great Britain

He retired from active service in July, 1920 In 1919 he was made a K B E He had taken the degree of Sc D at Cambridge, and among the honours he had received was the degree of LL D (Toronto) He was Wollaston medallist of the Geological Society in 1919 an honorary member of the Institution of Mining Engineers the Institution of Mining and Metallurgy the Institution of Petroleum Technologists and a corresponding member of the Geological Society of Belgium

On his retirement his geological activities by no means ceased but he thoroughly enjoyed country life and was greatly attached to his Oxfordshire home His final contribution to geological literature was a handbook to the Geological Model of the Goring district exhibited in the Museum of Practical Geology in which he showed his keen appreciation of the relation between physiographical features and the stratigraphy and tectonics of the district He had been familiar with that ground for many years as in 1891 he had published a brief paper on the Phosphatic Chalk of Taplow

There can be no doubt that when he elected to become a field geologist Sir Aubrey Strahan made a fortunate choice of a career Loving the life of the open air physically active and gifted with a keen appreciation of natural scenery he enjoyed his work thoroughly Through his whole life he was immersed in the study of British stratigraphy and his other intellectual interests were subordinated He had no great appreciation of palæontology or petrography which he left to his highly trained collaborators but he was an excellent judge of a map To some extent this restricted his geological outlook but it was compensated by the special excellence of his cartographical work Painstaking methodical and diligent he was much trusted by his colleagues His admirable administrative abilities soon brought him to the fore and involved him in much activity that was not strictly scientific

On committees Strahan was a very valuable member as his judgment was shrewd and dispassionate The economic applications of scientific research appealed to him strongly and he readily won the confidence of engineers and industrialists with whom he came in contact Personally he was rather reserved and undemonstrative at first but those who knew him well valued his friendship very highly He was absolutely free from any trace of exaggeration and his statements were carefully weighed concise and strictly relevant In private life he was a charming host and companion with an inexhaustible fund of reminiscences of British geologists and scientific men of all kinds and classes A day s tramp with him in country that he knew well was an experience sure to be remembered with deep appreciation and by the gleam of the firelight after a hard day s work all the genial and friendly side of his personality would come out rendering his conversation unforgettable to any young geologist who had the privilege of his society

J S F

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